STUDIA UNIVERSITATIS
BABEŞ-BOLYAI

Halophiles 2019

12th International Conference on Halophilic Microorganisms
June 24-28, 2019, Cluj-Napoca, Romania

BIOLOGIA

1/2019

STUDIA
UNIVERSITATIS BABEŞ-BOLYAI
BIOLOGIA

12TH CONFERENCE ON
HALOPHILIC MICROORGANISMS
"HALOPHILES 2019"

24-28
JUNE
CLUJ-NAPOCA

BOOK OF ABSTRACTS

1 / 2019
January – June

Halophiles 2019
12th International Conference on Halophilic Microorganisms
June 24-28, 2019, Cluj-Napoca, Romania

COMMITTEES

SCIENTIFIC ADVISORY BOARD (ALPHABETICAL LIST)

Dr. **Thorsten Allers**
University of Nottingham,
UNITED KINGDOM

Dr. **Josefa Antón**
University of Alicante, SPAIN

Dr. **Mădălin Enache**
Institute of Biology Bucharest,
Romanian Academy, ROMANIA

Prof. **Erwin A. Galinski**
Institute of Microbiology &
Biotechnology,
University of Bonn, GERMANY

Dr. **John E. Hallsworth**
Queen's University, Belfast,
UNITED KINGDOM

Prof. **Julie A. Maupin-Furlow**
University of Florida, USA

Dr. **Hiroaki Minegishi**
Research Institute of Industrial
Technology, Toyo University, JAPAN

Prof. **Aharon Oren**
The Hebrew University of Jerusalem,
ISRAEL

Dr. **Ramon Rosselló-Móra**
Grup de Microbiologia Marina
IMEDEA (CSIC-UIB), SPAIN

Prof. **Antonio Ventosa**
University of Sevilla, SPAIN

INVITED SPEAKERS (ALPHABETICAL LIST)

Prof. **Bonnie K. Baxter**
Great Salt Lake Institute,
Westminster College, USA

Dr. **Kathleen C. Benison**
West Virginia University, USA

Prof. **Erhard Bremer**
University of Marburg, GERMANY

Prof. **Rick Cavicchioli**
University of Sydney, New South Wales,
AUSTRALIA

Prof. **Terry McGenity**
University of Essex, UNITED KINGDOM

Dr. **Maria S. Muntyan**
Lomonosov Moscow State University,
RUSSIA

Prof. **Mechthild Pohlschröder**
University of Pennsylvania, USA

Prof. **Amy K. Schmid**
Duke University, USA

Dr. **Jocelyne DiRuggiero**
Johns Hopkins University, USA

Jun. Prof. Dr. **Sabine Filker**
University of Kaiserslautern, GERMANY

Prof. **Nina Gunde-Cimerman**
University of Ljubljana, SLOVENIA

Dr. **Hans-Jörg Kunte**
Biologische Materialschädigung und Referenzorganismen, GERMANY

Prof. **Francisco Rodriguez-Valera**
Universidad Miguel Hernandez, San Juan de Alicante, SPAIN

Prof. **Jörg Soppa**
Goethe-Universität Frankfurt am Main,
GERMANY

Dr. **Michail M. Yakimov**
Institute for Coastal Marine Environment, ITALY

ORGANIZING COMMITTEE

Prof. **Horia L. Banciu**
Babeș-Bolyai University

Diana F. Bogdan
Babeș-Bolyai University

Tiberiu Szöke-Nagy
National Institute for Research and Development of Isotopic and Molecular Technologies (NIRDIMT)

Sebastian A. Porav
National Institute for Research and Development of Isotopic and Molecular Technologies (NIRDIMT)

Valentin Rauca
Babeș-Bolyai University

Lecturer Dr. **Karina P. Battes**
Babeș-Bolyai University

Dr. **Andreea Baricz**
NIRDBS, Institute of Biological Research Cluj-Napoca

Prof. **Bálint Markó**
Babeș-Bolyai University

EXECUTIVE EDITORS STUDIA UBB BIOLOGIA

Lecturer Dr. **Karina P. Battes**
Babeș-Bolyai University

Lecturer Dr. **Mirela Cîmpean**
Babeș-Bolyai University

studia.ubb.biologia@gmail.com

SPONSORS

farmacia DONA

FEDERATION OF EUROPEAN MICROBIOLOGICAL SOCIETIES
FEMS

International Society for Salt Lake Research
Promoting the study, management, use, and conservation of salt lakes

DANUBIUS HOTELS GROUP

SALINA TURDA
Poartă spre inima Transilvaniei

Dexter.com
Life Science For You

microorganisms
an open access journal by MDPI

Dialab Solutions®

BIOTECH
CONSUMABILE LABORATOR

Bio Zyme

SCIENTIFIC PARTNERS

UNIVERSITATEA BABEȘ-BOLYAI
BABEȘ-BOLYAI TUDOMÁNYEGYETEM
BABEȘ-BOLYAI UNIVERSITÄT
TRADITIO ET EXCELLENTIA

O SUTĂ DE ANI DE UNIVERSITATE ROMÂNEASCĂ CLUJ 1919 - 2019
UNIVERSITATEA BABEȘ-BOLYAI

FACULTATEA DE BIOLOGIE ȘI GEOLOGIE
UNIVERSITATEA BABEȘ-BOLYAI, CLUJ-NAPOCA

I.N.C.D.T.I.M.

OSpN

ISIS
INSTITUTE OF SCIENCE AND INNOVATION FOR SOCIETY

RSBI
Societatea Română de Bioinformatică

Societatea Română de Microbiologie

Babeș-Bolyai University of Cluj-Napoca
Faculty of Biology and Geology - Babeș-Bolyai University
Doctoral School of Integrative Biology - Babeș-Bolyai University
National Institute for Research and Development of Isotopic and Molecular Technologies - INCDTIM (NIRDIMT)
Student's Organization for Nature
Institute of Science and Innovation for Society
Romanian Society of Bioinformatics (RSBI)
Romanian Society for Microbiology (SRM)

PROGRAM

Monday, 24 June, 2019

14.00–18.00 *REGISTRATION [Grand Hotel Napoca, http://hotelnapoca.ro/]*

18.00–18.30 *Welcome to Halophiles 2019*

18.30–19.30 OPENING LECTURE: **Rick Cavicchioli**, "Antarctic hypersaline communities – *environmental 'omics' and laboratory studies*"

19.30–21.00 *Welcome Dinner [Grand Hotel Napoca]*

Tuesday, 25 June, 2019

8.00-8.30 *REGISTRATION [Grand Hotel Napoca]*

SESSION: DIVERSITY & ECOLOGY (I)

(CHAIRPERSON: FRANCISCO RODRIGUEZ-VALERA)

8.30-9.00 **Mădălin Enache**, "How salted is Romania?"

9.00-9.30 **Aharon Oren**, "Why wasn't the Dead Sea red in the first half of the 20th century?"

9.30-10.00 **Bonnie Baxter**, "Biogeography, halophiles, and avian mechanical carriers"

10.00-10.20 **Nikolaos Korovessis**, "The ecological importance of solar saltworks"

10.30-11.00 *COFFEE/TEA BREAK*

SESSION: DIVERSITY & ECOLOGY (II)

(CHAIRPERSON: MĂDĂLIN ENACHE)

11.00-11.30 **Jocelyne DiRuggiero**, "Halite microbiome dynamics"

11.30-12.00 **Antonio Ventosa**, "Hypersaline environments microbiome: soils *versus* salterns"

12.00-12.20 **Nicoletta Perini**, "Purple Spots on ancient parchments *vs* Red Heats on present-day leathers: the unveiled secret of the salt-cured hides"

12.20-12.40 **Andrea Cherkouk**, "Interaction of uranium with halophilic microorganisms"

12.40-13.00 **Noor-Ul-Huda Ghori**, "Uncovering the microbial interactome: The social life of extremophiles"

13.00-14.00 *LUNCH*

SESSION: DIVERSITY & ECOLOGY (III)

(CHAIRPERSON: BONNIE BAXTER)

14.00-14.30	**Terry J. McGenity**, “Life in a pinch of salt”
14.30-15.00	**Francisco Rodriguez-Valera**, “Marine-freshwater transitions require extensive changes in the proteome. What it takes to live without salt”
15.00-15.30	**Michail M. Yakimov**, “Microbial diversity in the deep-sea anaerobic hypersaline environments with emphasis on the role of anaerobic haloarchaea in C and S cycles”
15.30-16.00	*COFFEE/TEA BREAK*

SESSION: DIVERSITY & ECOLOGY (IV)

(CHAIR PERSON: JOSEFA ANTÓN)

16.00-16.30	**Kathleen Benison**, "Integrating geology and microbiology to understand preservation of extremophiles in salt minerals"
16.30-17.00	**Mike Dyall-Smith**, “Haloviruses: the bad, the worse and the surprising”
17.00-17.20	**Raeid Abed**, “Structural-functional analysis of extremely hypersaline microbial mats from the Arabian Peninsula”
17.20-17.40	**Fernando Santos**, “Metaviromics asks, metaviroproteomics answers”
17.40-18.00	Sponsor presentation: **Ionuț Oprea** (*Dextercom SRL*), “Microbial community analysis”
18.30-20.30	*SOCIAL PROGRAM: CLUJ-NAPOCA CITY TOUR*

Wednesday, 26 June, 2019

SESSION: BIOCHEMISTRY & PHYSIOLOGY (I)

(CHAIRPERSON: MIKE DYALL-SMITH)

8.30-9.00	**Maria S. Muntyan**, “Bioenergetics of haloalkaliphiles”
9.00-9.30	**Erhard Bremer**, “The extremolytes ectoine and hydroxyectoine: import, synthesis and genetic regulation in response to osmotic stress”
9.30 -10.00	**Hans-Jörg Kunte**, “Effect of ectoine on DNA: Mechanisms of interaction and protection”
10.00-10.20	**Laura Czech**, “Synthesis, uptake and excretion of ectoines in the alphaproteobacteria *Hyphomonas neptunium* and *Novosphingobium* sp. LH128”
10.30-11.00	*COFFEE/ TEA BREAK*

SESSION: BIOCHEMISTRY & PHYSIOLOGY (II)
(CHAIRPERSON: ERHARD BREMER)

11.00-11.30	**Mechthild Pohlschröder**, “C-terminal lipid-anchoring of *Haloferax volcanii* surface proteins”
11.30-12.00	**Amy Schmid**, “Co-evolution of transcription and metabolic networks in halophilic archaea”
12.00-12.20	**Uri Gophna**, “CRISPR-Cas systems in haloarchaea more than just antiviral defense?”
12.20-12.40	**Thomas Thompson**, “Quorum sensing: signaling from haloarchaea to bacteria”
12.40-13.00	**Hosein Geraili** & Ana Vila Verde, “Molecular mechanisms behind protein halotolerance”
13.00-14.00	*LUNCH*

SESSION: ASTROBIOLOGY
(CHAIRPERSON: KATHLEEN BENISON)

14.00-14.30	**John E. Hallsworth**, “Saturated salt is not a limit for life on Earth”
14.30-15.00	Scott Perl & **Bonnie Baxter**, “Mineral preservation and photoprotective attributes of halophilic bacteria and archaea in modern and Permian lakebed environments”
15.00-15.30	**Jan Jehlička**, “Raman spectroscopy to detect biomarkers of halophiles in salts of evaporites: from natural colonisations to synthetic inclusions”
15.30-16.00	*COFFEE/TEA BREAK*

SESSION: GENETICS & EVOLUTION
(CHAIRPERSON: TERRY J. MCGENITY)

16.00-16.30	**Thorsten Allers**, “Evolution of chromosome architecture in *Haloferax volcanii*”
16.30-17.00	**Jörg Soppa**, “Polyploidy in halophilic Archaea: regulation, evolutionary advantages, and gene conversion”
17.00-17.30	**Josefa Antón**, “Viral evolution and diversification: what have we learnt from *Salinibacter* and its viruses?”
17.30-17.50	**Rafael R. de la Haba**, “First complete genome sequence of a *Salinivibrio* member and phylogenomics of the whole genus”
18.00-19.00	JOINT MEETING OF THE ICSP SUBCOMMITTEES ON ‘Taxonomy of Halobacteria’ and ‘Taxonomy of *Halomonadaceae*’

Thursday, 27 June, 2019

SESSION: DIVERSITY OF HALOPHILIC EUKARYOTES

(CHAIRPERSON: AHARON OREN)

8.30-9.00	**Sabine Filker**, "Diversity patterns and haloadaptation strategies of protists"
9.00-9.20	**Andrey Plotnikov**, "The diversity of protist communities in inland saline lakes and rivers of the Volga-Ural steppes (Russia)"
9.20-9.50	**Nina Gunde-Cimerman**, "The maverick fungal genus Wallemia – from halotolerant *W. mellicola* to halophilic and chaotolerant *W. ichthyophaga*"
10.00-10.20	**Cene Gostinčar**, "On generalists and specialists among halotolerant fungi"
10.30-11.00	*COFFEE/TEA BREAK*

SESSION: BIOTECHNOLOGY

(CHAIRPERSON: ERWIN GALINSKI)

11.00-11.20	**Gabriela Teodosiu**, "Biotechnological potential of halophilic archaea isolated from Romanian extreme habitats"
11.20-11.40	**Bhakti Salgaonkar**, "Coproduction of alpha amylase and polyhydroxyalkanoate by *Halococcus* strain GUVSC8"
11.40-12.00	**Sanket Gaonkar**, "Halo-thermophilic protease from Halococcus *agarilyticus* GUGFAWS-3 (MF425611)"
12.00-12.20	**Ram Karan**, "Single amplified genomes from Red Sea brine pool extremophiles - a source of novel enzymes"
12.20-12.40	**Doriana M. Buda**, "Capacity of *Halomicrobium mukohataei* DSM 12886 to overcome silver-induced oxidative stress"
12.40-13.00	**Lukas Bethlehem**, "Heterologous low-salt production of ectoine and hydroxyectoine: is *Escherichia coli* the answer?"
13.00-14.00	*LUNCH*
14.00-14.20	**Jaimi Butler**, "Got culture? Halophiles for teachers, students and our communities"
14.30-17.00	*POSTER SESSION & Poster evaluation for ISSLR Best Poster Award*
17.00-17.45	CLOSING LECTURE: **Erwin A. Galinski**, "The story of ectoine"

18.00-18.30 *Closing Ceremony: ISSLR Best Poster & Best Oral Presentation Awards, Farewell speech*

19.00-22.30 *Social Event by Ansamblul „Mugurelul" and Orchestra Simfonică Universitară UBB; Gala Dinner [Grand Hotel Napoca]*

Friday, 28 June, 2019

8.30 - 20.00 *Field trips to Salina Turda (http://salinaturda.eu/) and Cheile Turzii (Turda Gorge)*

YEAR Volume 64 (LXIV) 2019
MONTH JUNE
ISSUE 1

STUDIA
UNIVERSITATIS BABEŞ-BOLYAI
BIOLOGIA

1

STUDIA UBB EDITORIAL OFFICE: B.P. Hașdeu no. 51, 400371 Cluj-Napoca, Romania,
Phone + 40 264 405352, www.studia.ubbcluj.ro

SUMAR – CONTENTS – SOMMAIRE – INHALT

ORAL PRESENTATION ABSTRACTS

OPENING LECTURE

SESSION: BIODIVERSITY

SESSION: ECOLOGY AND BIOGEOCHEMISTRY

SESSION: BIOCHEMISTRY AND PHYSIOLOGY

SESSION: GENETICS AND EVOLUTION

SESSION: ASTROBIOLOGY

SESSION: BIOTECHNOLOGY

SESSION: EDUCATION

CLOSING LECTURE

POSTER ABSTRACTS

SECTION: BIODIVERSITY

SECTION: ECOLOGY AND BIOGEOCHEMISTRY

SECTION: BIOCHEMISTRY AND PHYSIOLOGY

SECTION: GENETICS AND EVOLUTION

SECTION: BIOTECHNOLOGY

All authors are responsible for submitting manuscripts in comprehensible US or UK English and ensuring scientific accuracy.

Original pictures on front cover: The “***Halophiles 2019***” logo represents a section through a collapsed bell-shaped salt mine. The outline is filled with a colored gradient denoting the permanent stratification of water in most of the salt lakes located in the Transylvanian Basin.
© Graphics by Robi Farkas & Oana Țiu

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 19-23)

About Halophiles 2019

Horia L. Banciu[1,✉] and Aharon Oren[2]

[1]Department of Molecular Biology and Biotechnology, Babeș-Bolyai University, 400006 Cluj-Napoca, Romania; [2]The Institute of Life Sciences, The Hebrew University of Jerusalem, The Edmond J. Safra Campus, 9190401 Jerusalem, Israel; ✉***Corresponding author, E-mail: horia.banciu@ubbcluj.ro.***

Abstract

In the present communication, we define the aim and settings of the 12th International Conference on Halophilic Microorganisms, held between 24-28 June at Cluj-Napoca, Romania. A brief chronological list of previous symposia and workshops on halophilic microorganisms held since 1978 is also provided alongside a short description of main benefits and perspectives of investigating the biology and diversity of halophiles.

Keywords: analogues for extraterrestrial habitats, bioprospecting, evolutionary biology, halophile genomics, ecology and metagenomics of saline environments, osmoadaptive mechanisms.

Aim of Halophiles 2019

The meeting intends to gather scientists around the world to share their enthusiasm and catalyze new lines of research in ecology, physiology, biodiversity, genetics, biochemistry, biophysics, biotechnology and evolutionary biology of salt-loving organisms or 'halophiles'.

Conference description

The 12th International Conference on Halophilic Microorganisms 'Halophiles 2019' will be held in Cluj-Napoca, Romania, from June 24 to 28, 2019 (http://www.halophiles.eu). The oral and poster sessions will cover topics related to taxonomy, biodiversity, biophysics, ecology, physiology, molecular biology, biochemistry, genetics, genomics, biotechnology, and other fields. There will also be a joint open meeting of the ICSP Subcommittees on 'Taxonomy of *Halobacteria*' and 'Taxonomy of *Halomonadaceae*'.

The relevance of the Halophiles 2019 conference

1. Short introduction.

Halophile biology covers many different topics, including basic aspects of the functioning of cells at high salt concentrations and low water activity, understanding the biodiversity of hypersaline ecosystems and their environmental health and sustainability, food science, biotechnological applications, and even space exploration and planetary protection.

2. Location of the conference.

The scientific community interested in life at high salt concentrations will meet again in Europe after three previous Halophiles meetings held in Asia (Beijing, China, 2010), North America (Storrs, CT, USA, 2013) and Central America (San Juan, Puerto Rico, 2016). Table 1, based in part on Oren (2011), presents a list of earlier conferences focused on the biology of halophiles.

Table 1. Symposia and workshops on halophilic microorganisms held since 1978. Updated from Oren (2011).

Dates	**Title**	**Organizers**	**Venue**	**FEMS supported**
May 14-19, 1978	Energetics and structure of halophilic microorganisms	S. R. Caplan, M. Ginzburg	Rehovot, Israel	
1981	EMBO Workshop on halophilic microorganisms	J. K. Lanyi	Ischia, Italy	
September 1-6, 1985	The molecular basis of haloadaptation in microorganisms	W. D. Grant, M. Kogut, K. Wegmann	Obermarchtal, Germany	●
March 23-28, 1986	Aspects of halophilism	H. Eisenberg	Jerusalem, Israel	
March 26 - April 5, 1989	Modern aspects of halophilism – the twelfth Edmond de Rothschild School in Molecular Biophysics	H. Eisenberg	Neve Ilan and Rehovot, Israel	
September 17-22, 1989	General & applied aspects of halophilic microorganisms	F. Rodríguez-Valera	Alicante, Spain	●
November 15-22, 1992	Halophilic bacteria: Research priorities and biotechnological potential for the 1990s	R. H. Vreeland	Williamsburg, VA, USA	
June 22-26, 1997	Microbiology and biogeochemistry of hypersaline environments	A. Oren	Jerusalem, Israel	
September 23-27, 2001	Halophiles 2001	A. Ventosa	Sevilla, Spain	●
September 4-9, 2004	Halophiles 2004	N. Gunde-Cimerman, A. Plemenitaš	Ljubljana, Slovenia	●
September 2-6, 2007	Halophiles 2007	T. J. McGenity	Colchester, UK	●
June 29 – July 3, 2010	Halophiles 2010	Y. Ma	Beijing, P.R. China	
June 23-27, 2013	Halophiles 2013	R. T. Papke	Storrs, CT, USA	
May 22-27, 2016	Halophiles 2016	R. Montalvo-Rodríguez	San Juan, Puerto Rico	
June 24-28, 2019	Halophiles 2019	H. L. Banciu	Cluj-Napoca, Romania	●

The congress will be organized for the first time in Romania. The host city, Cluj-Napoca (NW Romania) is located nearby numerous saline lakes and salt mines derived from exploitation of massive Miocene deposits of evaporites underlying the Transylvanian Basin (Alexe *et al.*, 2018).

Several Romanian groups of scientists mainly based in Cluj-Napoca and Bucharest have developed a long-term systematic interest in exploring salt lake ecosystems located in the Transylvanian Basin and SE Romania. Relevant discoveries made in Romanian salt lakes were reported since the end of 19th century and the beginning of 20th century by E. C. Teodoresco who named and described the green algal genus *Dunaliella* (Teodoresco, 1905) and by E. Daday who in 1888 described the halotolerant cladoceran *Moina salina* (Negrea, 1984). Professor A. I. Maxim coined the term 'heliothermal' for heat-accumulating lakes, a phenomenon that he documented in a number of Transylvanian salt lakes (Maxim, 1929; 1931). Recently, salt lakes from south and central Romania were the sources for isolation of novel archaeal (e.g., *Haloferax prahovense*) (Enache *et al.*, 2007) and bacterial taxa (e.g., *Rhodococcus sovatensis*) (Táncsics *et al.*, 2017). Additionally, microbial communities in the water column and sediments of permanently stratified hypersaline Transylvanian lakes were explored by next-generation sequencing, demonstrating the presence of a surprisingly high diversity of uncultivated microbes in such high-salt systems (Andrei *et al.*, 2015; 2017).

3. Benefits of understanding halophiles.

We all greatly benefit from the knowledge of these peculiar, salt-loving microbes in many fields. Halophiles are a rich source of extremozymes, many of which are stable in solvents and may find industrial applications (Oren, 2010; Yin *et al.*, 2015). While halophilic consortia have long been used in fermentative processing of food products such as in Asian fish sauce preparations, currently there are large-scale exploitations of the green alga *Dunaliella* for the production of β-carotene (used as food additive) and the bacterium *Halomonas* for the production of ectoine (as component of cosmetics and pharmaceutical formulations) (Oren, 2010). Besides their increasing biotechnological prospects, halophilic microorganisms are exciting models to explore both the physiological and biochemical adaptations to high salinity (and, implicitly, to low water activity) (Grant, 2004) and the molecular mechanisms involved in DNA replication, gene expression, genomic evolution and speciation. For example, the halophilic archaea *Haloferax volcanii* and *Haloferax mediterranei* were among first organisms in which the CRISPR/Cas system was detected (Mojica *et al.*, 1995), being an exciting example of heredity of acquired characteristics in biology. Finally, due to their high diversity and their wide repertoire of metabolic capabilities, halophilic microbes are intensively scrutinized as models of early life forms and for traits that may be found in hypothetical inhabitants of extraterrestrial, salt-rich habitats (Litchfield, 1998; Gómez *et al.*, 2012).

Acknowledgements. We gratefully acknowledge FEMS for supporting the Halophiles 2019 conference by FEMS Meeting Organizer Grant - FEMS-GO-2018-091. We also acknolwedge the grant of the Romanian National Authority for Scientific Research, CNCS–UEFISCDI, project number PN-III-P4-ID-PCE-2016-0303.

References

Alexe, M., Șerban, G., Baricz, A., Andrei, A. Ș., Cristea, A., Battes, K. P., Cîmpean, M., Momeu, L., Muntean, V., Porav, S. A., & Banciu, H. L. (2018). Limnology and plankton diversity of salt lakes from Transylvanian Basin (Romania): A review. *J Limnol* **77**: 17-34.

Andrei, A. Ș., Robeson II, M. S., Baricz, A., Coman, C., Muntean, V., Ionescu, A., Etiope, G., Alexe, M., Sicora, C. I., Podar, M., & Banciu, H. L. (2015). Contrasting taxonomic stratification of microbial communities in two hypersaline meromictic lakes. *ISME J* **9**: 2642-2656.

Andrei, A. Ş., Baricz, A., Robeson, M. S., Păuşan, M. R., Tămaş, T., Chiriac, C., Szekeres, E., Barbu-Tudoran, L., Levei,, E. A., Coman, C. Podar, M., & Banciu, H. L. (2017). Hypersaline sapropels act as hotspots for microbial dark matter. *Sci Rep* **7**: 6150.

Enache, M., Itoh, T., Kamekura, M., Teodosiu, G., & Dumitru, L. (2007). *Haloferax prahovense* sp. nov., an extremely halophilic archaeon isolated from a Romanian salt lake. *Int J Syst Evol Microbiol* **57**: 393-397.

Grant, W. D. (2004). Life at low water activity. *Phil Trans R Soc Lond B Biol Sci* **359**: 1249-1267.

Gómez, F., Rodríguez-Manfredi, J. A., Rodríguez, N., Fernández-Sampedro, M., Caballero-Castrejón, F. J., & Amils, R. (2012). Habitability: where to look for life? Halophilic habitats: earth analogs to study Mars habitability. *Planet Space Sci* **68**: 48-55.

Litchfield, C. D. (1998). Survival strategies for microorganisms in hypersaline environments and their relevance to life on early Mars. *Meteor Planet Sci* **33**: 813-819.

Maxim, I. A. (1929). Contribution to explaining salt waters warm-up phenomenon in Transylvania. Lakes in Sovata. [Article in Romanian]. *Rev Muz Geol-Mineral Univ Cluj* **3**: 49-83.

Maxim, I. A. (1931). Kontribution zur Erklärung des Erwärmungsprozesses der Salzteiche von Siebenbürgen. II. Die Teiche von Ocna-Sibiului. *Rev Muz Geol-Mineral Univ Cluj* **4**: 47-111.

Mojica, F. J. M., Ferrer, C., Juez, G., & Rodriguez-Valera, F. (1995). Long stretches of short tandem repeats are present in the largest replicons of the Archaea *Haloferax mediterranei* and *Haloferax volcanii* and could be involved in replicon partitioning. *Mol Microbiol* **17**: 85-93.

Negrea, S. (1984). Redescription de *Moina salina* Daday, 1888 (Cladocera, Moinidae) d'après des exemplaires trouvés en terra typica. *Crustaceana* **47**: 83-97.

Oren, A. (2010). Industrial and environmental applications of halophilic microorganisms. *Environ Technol* **31**: 825-834.

Oren, A. (2011). A short history of the symposia on halophilic microorganisms: from Rehovot 1978 to Beijing 2010. In: Ventosa, A., Oren, A., and Ma, Y. (eds.) Halophiles and hypersaline environments. Current Research and Future Trends. Springer, Heidelberg, pp. 373-382.

Táncsics, A., Máthé, I., Benedek, T., Tóth, E. M., Atasayar, E., Spröer, C., Márialigeti, K., Felföldi, T., & Kriszt, B. (2017). *Rhodococcus sovatensis* sp. nov., an actinomycete isolated from the hypersaline and heliothermal Lake Ursu. *Int J Syst Evol Microbiol* **67**: 190-196.

Teodoresco, E.C. (1905). Organisation et développement du *Dunaliella*, nouveau genre de Volvocacée - Polyblepharidée. *Beih Bot Centralbl* **18**(Abt. 1): 215-232.

Yin, J., Chen, J. C., Wu, Q., & Chen, G. Q. (2015). Halophiles, coming stars for industrial biotechnology. *Biotechnol Adv* **33**: 1433-1442.

ORAL PRESENTATION ABSTRACTS

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 27)

Antarctic hypersaline communities – *environmental 'omics' and laboratory studies*

Ricardo Cavicchioli[1,✉]

[1] *School of Biotechnology and Biomolecular Sciences, UNSW Sydney, NSW 2052, Australia;*
✉ ***Corresponding author, E-mail: r.cavicchioli@unsw.edu.au.***

Abstract

Antarctic lakes are a treasure trove for the discovery of microbes with previously unknown properties. Marine-derived, hypersaline systems, including the coldest natural environment on Earth known to support life, are present in the Vestfold Hills and Rauer Islands regions of East Antarctica (near the Australian research station, Davis). The lakes support diverse archaea and viruses. In this talk I will describe hypothesis-based and serendipitous discoveries involving synergies realized through field and laboratory research. Environmental 'omic' approaches (metagenomics and metaproteomics) have been instrumental in learning about the ecology (e.g. community structure, interactions) and evolution (e.g. gene transfer, niche adaptation) of communities, with laboratory cultivation and manipulation of lake isolates enabling targetted assessments of physiology, metabolism and interactions (cell-cell and cell-virus). Laboratory experimentation using field samples has also proved lucrative for discovering unexpected mobile genetic elements and cellular interactions, with ecological relevance of the discoveries being realized through further interrogation of environmental 'omics' data. By studying Antarctic hypersaline communities we complement research efforts of lower-latitudinal environments, thereby expanding understanding of endemism, biogeography and the global halophile pan-genome.

Keywords: archaeal evolution, ecophysiological adaptation, gene exchange, haloarchaea, halovirus.

Acknowledgements. This work was suported by the Australian Research Council.

Halite microbiome dynamics

Gherman Uritskiy[1], Diego Gelsinger[1], Adam Munn[1] and
Jocelyne DiRuggiero[1,✉]

[1]*Johns Hopkins University, 3400 N. Charles Steet, Baltimore, USA;*
✉***Corresponding author, E-mail: jdiruggiero@jhu.edu.***

Abstract
Microorganisms are essential to our biosphere and, as such, understanding the mechanisms underlying their resilience is essential to predict the impact of climate change on Earth's ecosystems. In the most arid deserts on Earth, endolithic microbial communities colonize the rocks' interior as a survival strategy; because of their extreme nature, these communities are particularly sensitive to environmental changes. Over a 4-year longitudinal study, using shotgun metagenomic sequencing, we characterize the temporal response of a highly specialized community inhabiting halite (salt rocks) in the Atacama Desert, to a catastrophic rainfall. Halite communities harbor mostly members of the Archaea, unique cyanobacteria, diverse heterotrophic bacteria, and a novel type of algae (Crits-Christoph *et al.*, 2016). Our results suggest an initial response to the rain in which the community entered an unstable intermediate state after stochastic niche re-colonization, resulting in broad predicted protein adaptations to increased water availability. In contrast, during recovery, the community returned to its former functional potential by a gradual shift in abundances of the newly acquired taxa (Uritskiy *et al.*, 2019). The characterization these two modes of community response could potentially be applied to other ecosystems, providing a theoretical framework for prediction of taxonomic and functional flux following environmental changes. At the molecular level, we discovered hundreds of small RNAs with potential regulatory roles and taxonomically assigned to diverse members of the halite community. Our data demonstrate that field experiments, linking environmental variation with changes in RNA pools, have potential to provide new insights into environmental sensing and response in natural microbial communities.

Keywords: halite, microbial communities, sRNAs, stress response.

Acknowledgements. This work was suported by grants NNX15AP18G and NNX15AK57G from NASA, DEB1556574 from the NSF

References

Crits-Christoph, A., Gelsinger, D.R., Ma, B., Wierzchos, J., Ravel, J., Davila, A., Casero, M. C., & DiRuggiero, J. (2016). Functional interactions of archaea, bacteria and viruses in a hypersaline endolithic community. *Env Microbiol* **18**: 2064-2077.

Uritskiy, G., Getsin, S., Munn, A., Gomez-Silva, B., Davila, A., Glass, B., Taylor, J., & DiRuggiero, J. (2019). Halophilic microbial community compositional shift after a rare rainfall in the Atacama Desert. *bioRxiv* 442525; doi: https://doi.org/10.1101/442525.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 30)

Hypersaline environments microbiome: soils versus salterns

Antonio Ventosa[1], Blanca Vera-Gargallo[1], Mª José León[1],
Ana Durán-Viseras[1], Rafael R. de la Haba[1] and Cristina Sánchez-Porro[1]

[1]Department of Microbiology and Parasitology, University of Sevilla, 41012 Sevilla, Spain; ✉***Corresponding author, E-mail: ventosa@us.es.***

Abstract

Hypersaline environments comprise aquatic and terrestrial habitats. During recent years the prokaryotic diversity and ecology of aquatic hypersaline environments has been addressed by metagenomic studies (Ventosa *et al*., 2015). In contrast, a limited number of studies on the microbial diversity of saline soils have been carried out, and they have been mainly focused on either *Bacteria* or *Archaea*. To improve our understanding of the prokaryotic community structure in saline soils, we studied recently the phylogenetic diversity and metabolic potential of the prokaryotic community of hypersaline soils from the Odiel saltmarshes (Spain) by metagenomics. Comparative analyses of these saline soils metagenomic databases with available metagenomic databases from salterns ponds allowed further identification of unique and shared traits of the prokaryotic communities dwelling in these habitats. Saline soils harbored a more diverse prokaryotic community and, in contrast to their aquatic counterparts, comprised sequences related to both known halophiles and taxa without known halophilic or halotolerant representatives, which reflects the physical heterogeneity of the soil matrix. The soils studied harbored unique communities mainly composed of halophilic taxa from the phyla *Euryarchaeota, Proteobacteria, Balneolaeota, Bacteroidetes* and *Rhodothermaeota* (Vera-Gargallo *et al*., 2018; 2019).

Keywords: hypersaline habitats, metagenomics, prokaryotic diversity, salterns, saline soils.

Acknowledgements. This work was suported by project CGL2017-83385-P (Spanish Ministry of Economy and Competitiveness-MINECO) and by the Junta de Andalucia (BIO-213), both with FEDER funds.

References

Ventosa, A., de la Haba, R.R., & Sánchez-Porro, C. (2015). Microbial diversity of hypersaline environments: a metagenomic approach. *Curr Opin Microbiol* **25**: 80-87.

Vera-Gargallo, B., & Ventosa, A. (2018). Metagenomic insights into the phylogenetic and metabolic diversity of the prokaryotic community dwelling in hypersaline soils from the Odiel saltmarshes (SW Spain). *Genes* **9**: 152.

Vera-Gargallo, B., Chowdhury, T. R., Brown, J., Fansler S. J., Durán-Viseras, A., Sánchez-Porro, C., Bailey, V. L., Jansson, J. K., & Ventosa, A. (2019). Spatial distribution of prokaryotic communities in hyerpsaline soils. *Sci Rep* **9**: 1769.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 31-32)

High resilience and resistance of solar saltern microbial assemblages exposed to different environmental pressures

Tomeu Viver[1, ✉], Luis H. Orellana[2], Sara Díaz[1], Mercedes Urdiain[1], María Dolores Ramos-Barbero[3], José E. González-Pastor[4], Aharon Oren[5], Rudolf Amann[6], Josefa Antón[3], Konstantinos T. Konstantinidis[2] and Ramon Rosselló-Móra[1]

[1]Mediterranean Institute for Advanced Studies (IMEDEA), Department of Animal and Microbial Biodiversity, Marine Microbiology Group, Esporles, Spain; [2]Georgia Institute of Technology, School of Civil & Environmental Engineering, Atlanta, Georgia, USA; [3]University of Alicante, Department of Physiology, Genetics and Microbiolgoy, Alicante, Spain; [4]Centro de Astrobiología, Department of Molecular Evolution, Madrid, Spain; [5]The Hebrew University of Jerusalem, The Institute of Life Sciences, Department of Plant and Evironmental Science, Jerusalem, Israel; [6]Max-Planck-Institute für Marine Mikrobiologie, Department of Molecular Ecology, Bremen, Germany; ✉***Corresponding author, E-mail: tviver@imedea.uib-csic.es.***

Abstract

Solar saltern ponds of the Es Trenc (Mallorca-Spain), with crystallizer ponds containing about 15m^3 of brine, represents an ideal setups for in situ mesocosm studies. Here, the influence of light intensities and the effect of sudden dilution were followed using metagenomics. The results indicated that light intensity and salinity are the deterministic factors driving the establishment of well-adapted saltern microbial communities. Specifically, under high-irradiation and high-salinity conditions, highly resilient microbial communities were mainly dominated by the well-known *Haloquadratum* spp. and *Salinibacter* spp. On the other hand, different microbial populations less resistant to changes dominated under low-irradiation, with a yet unclassified haloarchaeon and *Spiribacter*-like bacteria representing the dominant species. In addition to these environmental pressures, a sudden decrease of salinity from 34% to 12% highlighted a major sensitivity of the archaeal but not the bacterial fractions. Once the salinity recovered to saturation levels, the highly resilient and well established community dominated by *Haloquadratum* spp. and *Salinibacter* spp. reestablished. Additional shifts observed during salt dilution were related to changes in primary producers and virus predation that influenced the dynamics of the microbial communities. Collectively, our results support that environmental factors assayed here deterministically select the organisms that dominate saltern the microbial communities.

Keywords: hypersaline environments, resilience, temporal series.

Acknowledgements. This study was funded by the Spanish Ministry of Economy projects CGL2012-39627-C03-03 and CLG2015_66686-C3-1-P (to RRM), CGL2015_66686-C3-3-P (to JA) and CGL2015_66686-C3-2-P (to JEGP) which were also supported with European Regional Development Fund (FEDER) funds.RA was funded by the Max Planck Society. KTK's research was supported, in part, by the U.S. National Science Foundation (Award No. 1831582). TVP received a pre-doctoral fellowship (Nr. BES-2013-064420) from the Spanish Government Ministry for Finance and Competition.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 33)

First complete genome sequence of a *Salinivibrio* member and phylogenomics of the whole genus

Rafael R. de la Haba[1,✉], Clara López-Hermoso[1], Cristina Sánchez-Porro[1], Konstantinos T. Konstantinidis[2] and Antonio Ventosa[1]

[1]*University of Sevilla, Faculty of Pharmacy, Department of Microbiology and Parasitology, Prof. García González 2, Sevilla, Spain;* [2]*Georgia Institute of Technology, School of Civil and Environmental Engineering, 311 Ferst Drive, 3224 ES&T, Atlanta, Georgia, USA;*
✉***Corresponding author, E-mail: rrh@us.es.***

Abstract

Salinivibrios are moderately halophilic bacteria that inhabit hypersaline environments, brines and salty meats. Our group has recently sequenced 36 draft genomes including type and reference strains within the genus *Salinivibrio* (López-Hermoso *et al.*, 2017). Nevertheless, no complete genome sequence is currently available for this genus. Therefore, this study was focused on the complete genome reconstruction of the type strain of *S. kushneri* AL184^T and on the detailed phylogenomic analysis of the available *Salinivibrio* genomes. A PacBio library with 10 kbp insert size was constructed and a 350X coverage was achieved. The genome of *S. kushneri* AL184^T consists of two circular chromosomes containing 2,840,906 bp and 602,384 bp, 2,524 and 531 CDS, and a DNA G+C content of 50.9 % and 50.1 %, respectively. This complete genome was used as a reference for synteny analyses and contig arrangement of the other *Salnivibrio* type strain genomes. Phylogenomics of this genus was performed by using the core orthologous gene set identified by shared reciprocal best matches. This analysis allowed the proper affiliation of the *Salinivibrio* strains to a validly published species name.

Keywords: complete genome, phylogenomics, *Salinivibrio*, synteny, taxonomy.

Acknowledgements. This work was suported by projects CGL2017-83385-P (MINECO/FEDER, Spain) and by Junta de Andalucía (BIO-213, Spain).

Reference

López-Hermoso, C., de la Haba, R. R., Sánchez-Porro, C., Bayliss, S. C., Feil, E. J., & Ventosa, A. (2017). Draft genome sequences of *Salinivibrio proteolyticus*, *Salinivibrio sharmensis*, *Salinivibrio siamensis*, *Salinivibrio costicola* subsp. *alcaliphilus*, *Salinivibrio costicola* subsp. *vallismortis*, and 29 new isolates belonging to the genus *Salinivibrio*. *Genome Announc* **5**: e00244-17.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 34-35)

Diversity patterns and haloadaptation strategies of protists

Sabine Filker[1,✉], Lea Weinisch[1] and Steffen Kühner[1]

[1]*University of Kaiserslautern, Erwin-Schroedinger-St. 14, 67663 Kaiserslautern, Germany;*
✉***Corresponding author, E-mail: filker@rhrk.uni-kl.de.***

Abstract

The identification of environmental barriers that govern species distribution is a fundamental concern in ecology. Salt was previously identified as a major transition boundary for micro- and macroorganisms alike, selecting for organisms adapted to this environmental stressor and probably also preventing their dispersal. The salinities causing species turnover in protistan communities, however, remained unknown. We analysed protistan plankton community structures in various salt ponds from different geographic regions. Salinity in these habitats ranged between 4 to >40%. Partitioning of diversity pointed to a niche differentiation, suggesting distinct salinity classes defining the boundaries for protistan community turnover. Regardless of their geographic origin, protistan communities in these salinity categories displayed different taxonomic memberships. Laboratory experiments using halophile ciliates were conducted to investigate physiological constraints for the observed protistan community turnover in hypersaline waters. Using proton nuclear magnetic resonance spectroscopy, the use of two compatible solutes to combat high-salt conditions could be shown, and, furthermore, significant positive correlations of intracellular concentrations of the compatible solutes and external salinity could be revealed. This is the first experimental evidence that heterotrophic protists use the salt-out strategy as haloadaptation. However, the observation of varying relative proportions of compatible solutes within the four ciliates points to slight differences in haloadaptive strategies by regulatory action of the ciliates. Based on this finding, we provide an explanatory hypothesis for the distribution of protistan diversity along salinity gradients.

Keywords: ciliates, compatible solutes, haloadaptation, protists, transition boundaries

References

Filker, S., Forster, D., Weinisch, L., Mora Ruiz, M., González, B., Farías, M., Rosselló-Móra, R., & Stoeck, T. (2017). Transition boundaries for protistan species turnover in hypersaline waters of different biogeographic regions. *Environ Microbiol* **19**: 3186-3200.

Weinisch, L., Kühner, S., Roth, R., Grimm, M., Roth, T., Netz, D. J. A., Pierik, A. & Filker, S., (2018). Identification of osmoadaptive strategies in the halophile, heterotrophic ciliate *Schmidingerothrix salinarum*. *PLoS Biol* **16**: e2003892.

Weinisch, L., Kirchner, I., Grimm, M., Kühner, S., Pierik, A., Roselló-Móra, R. & Filker, S., (2018). Glycine betaine and ectoine are the major compatible solutes used by four different halophilic heterotrophic ciliates. *Microb Ecol* **72**: 317-331.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 36)

The diversity of protist communities in inland saline lakes and rivers of the Volga-Ural steppes (Russia)

Andrey Plotnikov[1,✉], Elena Selivanova[1], Daria Poshvina[1] and
Yuri Khlopko[1]

[1]Institute for Cellular and Intracellular Symbiosis of the Ural Branch of the Russian Academy of Sciences, 460000, Pionerskaya st, 11, Orenburg, Russia;
✉***Corresponding author, E-mail: protoz@mail.ru.***

Abstract

Protists are ubiquitous aquatic unicellular eukaryotes playing a key role in microbial food webs of saline water ecosystems. However, there are few descriptions of the diversity of protist communities inhabiting inland saline water bodies. We analysed the taxonomic composition and diversity of protists in inland saline lakes and rivers located in the pre-Ural and the Lower Volga steppes (Russia). The salinity of the sites varied from 10 to 300 g/L. To characterize the protist diversity we used a comprehensive approach, including microscopic observation, cultivation, and high-throughput sequencing (HTS) of the 18S rRNA gene. The taxonomic structure of the protist communities in the studied lakes and rivers mainly depended on their salinity and geographic location. With increasing salinity, taxonomic richness and diversity of protists in the saline inland waters decreased at all taxonomic levels, from species up to phyla. Ordination of the eukaryotic communities by principal component analysis showed clustering of samples with high salinity (285-300 g/L), whereas other samples with lower salinity were not grouped clearly. A negligible number of shared genera in the studied water bodies indicated the unique taxonomic composition of each protist community. A high percentage of novel phylotypes was revealed. Extremely halotolerant and halophilic heterolobosean amoeboflagellates were found to be a specific heterotrophic component of the studied protist communities. Morphology, physiology, and molecular phylogeny of new heterolobosean taxa were characterised. The comprehensive approach, combining HTS of the 18S rRNA gene with classical protistological techniques, provides a valid estimation of protist diversity in inland saline water bodies.

Keywords: heterolobosea, high-throughput sequencing, inland saline water body, protists, protist community.

Acknowledgements. This work was suported by RFBR (17-04-02079, 17-04-00135).

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 37)

The maverick fungal genus *Wallemia* – from halotolerant *W. mellicola* to halophilic and chaotolerant *W. ichthyophaga*

Cene Gostinčar[1,2], Xiaohuan Sun[3,4], Janja Zajc [1,5], Polona Zalar[1],
Yuchong Tang[3,4], Chao Fang[3,4], Yonglun Luo[2,3,6], Zewei Song[3,4] and
Nina Gunde-Cimerman[1,✉]

[1]Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; [2]Lars Bolund Institute of Regenerative Medicine, BGI-Qingdao, Qingdao, China; [3]BGI-Shenzhen, Beishan Industrial Zone, Yantian, Shenzhen, China; [4]China National GeneBank, BGI-Shenzhen, Dapeng New District, Shenzhen, China; [5]National Institute of Biology, 1000 Ljubljana, Slovenia; [6]Department of Biomedicine, Aarhus University, Aarhus, Denmark;
✉***Corresponding author**, **E-mail: nina.gunde-cimerman@bf.uni-lj.si.***

Abstract

Most fungal species that form an integral part of microbial communities in different hypersaline environments around the world are halotolerant. They grow at salinities exceeding 2.9 M NaCl but also in media without salt. *Wallemia* spp. that until 2005 contained only the species *W. sebi* represents an exception. The genus now contains seven additional species: *W. ichthyophaga*, *W. muriae*, *W. mellicola*, *W. canadensis, W. tropicalis*, *W. hederae* and *W. peruviensis.* Three of them display obligate halophily. The rarest, and phylogenetically and morphologically most distinct species is *W. ichthyophaga*. It strongly prefers high concentrations of ionic over non-ionic solutes, it is able to grow in saturated NaCl, KCl and $MgSO_4$ solutions and even at around 2 M $MgCl_2$, explaining its isolation from magnesium rich bitterns. One of the most common and ubiquitous species of the genus is extremotolerant *W. mellicola*. It is distributed worldwide, in a range of habitats: soil, air, house dust, hypersaline water of solar salterns, salted, sugared and dried food products, seeds, straw, pollen and forest plants. Although halotolerant, it can grow at 4.1 M NaCl and 1.4 M $MgCl_2$. The first genome of *W. mellicola* was sequenced in 2012 and of *W. ichthyophaga* in 2013. To investigate the halotolerant/halophilic character and population characteristics of both species we now resequenced genomes of 22 *W. ichthyophaga* and of 25 *W. mellicola* strains, adding important information on this 500 million old basidiomycetous genus within sub-phylum Wallemiomycotina.

Keywords: basidiomycete, halophilic fungus, population genomics, recombination, specialist.

On generalists and specialists among halotolerant fungi

Cene Gostinčar[1,2,✉], Xiaohuan Sun[3,4], Janja Zajc [1,5], Polona Zalar[1], Martina Turk[1], Jerneja Zupančič[1], Yuchong Tang[3,4], Chao Fang[3,4], Yonglun Luo[2,3,6], Zewei Song[3,4] and Nina Gunde-Cimerman[1]

[1]Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; [2]Lars Bolund Institute of Regenerative Medicine, BGI-Qingdao, Qingdao, China; [3]BGI-Shenzhen, Beishan Industrial Zone, Yantian, Shenzhen, China; [4]China National GeneBank, BGI-Shenzhen, Dapeng New District, Shenzhen, China; [5]National Institute of Biology, 1000 Ljubljana, Slovenia; [6]Department of Biomedicine, Aarhus University, Aarhus, Denmark;
✉***Corresponding author**, **E-mail: cene.gostincar@bf.uni-lj.si.***

Abstract

Among the most frequently isolated halotolerant fungi is *Aureobasidium pullulans*, a species found in habitats as different as hypersaline water in the tropics and glacial ice of the polar regions. It is not only halotolerant, but polyextremotolerant, highly adaptable and nutritionally versatile. How can a species thrive in hypersaline water, but also survive conditions inside the Arctic glacier, on plant surface, in house dust and in other habitats? Or do generalist species in fact always harbour cryptic specialists, undetected by traditional phylogenetic studies, as indicated by some studies? We sequenced whole genomes of fifty strains of the black yeast *Aureobasidium pullulans* isolated from various habitats worldwide, as well as fifty genomes of *Aureobasidium melanogenum* and fifteen strains of *Aureobasidium subglaciale*. The genomes shared a relatively high degree of intraspecific variability. Nevertheless, the genomes of *A. pullulans* for example contained a redundancy of alkali metal cation transporters and this redundancy was highly conserved between the strains. Habitat generalists and habitat specialists differed in terms of the amount of genomic recombination and the existence of genetically defined subpopulations in the species. Certain fungi appear to be true generalists, with strains from a single recombining population inhabiting diverse habitats with no signs of specialisation for each of these habitats on the genomic level, while other, closely related species employ much more specialised ecological strategies.

Keywords: comparative genomics, fungi, generalists, population genomics

References

Gostinčar, C., Stajich, J. E., Zupančič, J., Zalar, P., & Gunde-Cimerman, N. (2018). Genomic evidence for intraspecific hybridization in a clonal and extremely halotolerant yeast. *BMC Genomics* **19**: 364

Gostinčar, C., Turk, M., Zajc, J., & Gunde-Cimerman, N. (2019). Fifty *Aureobasidium pullulans* genomes reveal a recombining polyextremotolerant generalist. *Environ Microbiol*, under review.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 39)

How salted is Romania?

Mădălin Enache[1,✉], Roxana Cojoc[1], Simona Neagu[1], Ioana Gomoiu[1], Ioana Lucaci[1], Robert Ruginescu[1], Gabriela Teodosiu[1], Larisa Florescu[1] and Mirela Moldoveanu[1]

[1]*Institute of Biology Bucharest of the Romanian Academy, 296 Splaiul Independentei, Bucharest, Romania;* ✉***Corresponding author, E-mail: madalin.enache@ibiol.ro.***

Abstract

Saline environments are widely distributed in the world as a consequence of the geological steps in the evolution of the Earth. A lot of saline lakes and salt deposit are also found on the Romania territory, the most representative being Ursu Lake, complex of saline lakes in Slanic Prahova or Ocna Sibiului and Salt Lake (Braila County). The salt deposits on the Romania territory have geological age from the periods of Triassic (in Moesic platform – south of Romania) and Miocene (Transylvania and Maramures areas). Over 300 salt massifs were identified in- or extra-Carpathian areas and in some places (Slanic Prahova, Praid, Sarata Monteoru) piercing the Earth in the form of salt „mountains" (Ciobanu, 2002). This work will be focused on the main results obtained in our laboratories in last years and the main investigated saline ecosystems will be presented. The studies on the salt lake complex and from salt deposit from Slanic Prahova area conducted to the isolation of a novel haloarchaea strain, namely *Haloferax prahovense* and have highlighted some relationship between microbial composition in lakes and deposit. Recently investigated saline lake Letea, located inner Danube Delta revealed a seasonally variable salinity of this ecosystem which is reflected in microbial communities not only halophilic one, but also involved in main biogeochemical cycles of nitrogen and sulphur. On the other hand, the halophilic microorganisms belonging to *Garicola* genus have been isolated from so called „unusual habitat for halophiles", namely mural painting from refectory of Hurezi Monastery.

Keywords: halophiles, pink coloration, saline ecosystem.

References

Ciobanu, D. (2002). Exploatarea sarii in perioada marilor migratii (sec. I – XIII e.n.) in spatiul carpato-dunarean. Ed. Alpha Buzau (in Romanian)

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 40-41)

Why wasn't the Dead Sea red in the first half of the 20th century?

Aharon Oren[1,✉], Rahel Elevi Bardavid[1], Lily Mana[1], Boaz Lazar[2],
Tanya Rivlin[3] and Ittai Gavrieli[4]

[1]The Hebrew University of Jerusalem, 9190401 Jerusalem, Israel; [2]The Institute of Earth Sciences, The Hebrew University of Jerusalem, 9190401 Jerusalem, Israel; [3]The Interuniversity Institute for Marine Sciences in Eilat, Eilat 8810302, Israel; [4]The Geological Survey of Israel, Yesha'ayahu Leibowitz 32, Jerusalem 9371234, Israel;
✉Corresponding author, E-mail: aharon.oren@mail.huji.ac.il.

Abstract

During the 19th century and the first decades of the 20th century, the Dead Sea was considered sterile. Only in the 1930s was the lake shown to harbor microbial life. Blooms of unicellular algae (*Dunaliella*) and halophilic Archaea (*Halobacteria*) were observed in 1980 and 1992, coloring the lake red (Oren, 1997). The question must be asked why such blooms were never reported earlier, despite the fact that the surface water salinity was sufficiently low for microbial proliferation. Phosphate is the limiting nutrient for primary production in the Dead Sea. We here show that phosphate likely was scavenged by gypsum that during earlier times precipitated from the lake's water column. Nowadays very little sulfate is left in the Dead Sea (< 5 mM). Significant amounts of gypsum are no longer formed, so that phosphate that enters with floodwaters in rainy years or with the limited base flow remains available and can trigger microbial blooms. Implementation of the planned Red Sea - Dead Sea water conveyance program to restore the shrinking Dead Sea by inflow of massive amounts of water from the Red Sea (Oren et al., 2004; Glausiusz, 2010) will again lead to massive precipitation of gypsum due to the high sulfate content of Red Sea water that will mix in the calcium-rich Dead Sea brine. Despite the expected dilution of the surface water, phosphate scavenging is then expected to deplete the lake of the limiting nutrient, making the chance of dense algal and archaeal blooms much smaller than predicted earlier.

Keywords: Dead Sea, *Dunaliella*, gypsum, *Halobacteria*, phosphate.

References

Oren, A. (1997). Microbiological studies in the Dead Sea: 1892-1992, In: Niemi T., Ben-Avraham Z., Gat, J.R. (eds.) *The Dead Sea - The Lake and its Setting.* Oxford University Press, pp. 205-213. New York, USA.

Oren, A., Gavrieli, I., Gavrieli, J., Lati, J., Kohen, M., Aharoni, M. (2004). Biological effects of dilution of Dead Sea water with seawater: implications for the planning of the Red Sea – Dead Sea "Peace Conduit". *J Mar Syst* **46**: 121-131.

Glausiusz, J. (2010). New life for the Dead Sea? *Nature* **464**: 1118-1120.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 42)

Biogeography, halophiles, and avian mechanical carriers

Bex L. Kemp[1], Erin M. Tabish[1], Adam J. Wolford[1], Daniel L. Jones[1],
Jaimi K. Butler[1] and Bonnie K. Baxter[1,]✉

[1]*Great Salt Lake Institute, Westminster College, 1840 South 1300 East, Salt Lake City Utah, USA, 84105;* ✉***Corresponding author, E-mail: bbaxter@westminstercollege.edu.***

Abstract

Halophilic archaea inhabit hypersaline ecosystems globally, and genetically similar strains have been found in locales that are geographically isolated from one another. We sought to test the hypothesis that small salt crystals harboring halophilic archaea could be carried on bird feathers and that bird migration is a driving force of these distributions. In this study, we discovered that the American White Pelicans (AWPE) at Great Salt Lake soak in the hypersaline brine and accumulate salt crystals (halite) on their feathers. We cultured halophilic archaea from AWPE feathers and halite crystals. The microorganisms isolated from the lakeshore crystals were restricted to two genera: *Halorubrum* and *Haloarcula,* however, archaea from the feathers were strictly *Haloarcula*. We compared partial DNA sequence of the 16S rRNA gene from our cultivars with that of similar strains in the GenBank database. To understand the biogeography of genetically similar halophilic archaea, we studied the geographical locations of the sampling sites of the closest-matched species. An analysis of the environmental factors of each site pointed to salinity as the most important factor for selection. The geography of the sites was consistent with the location of the sub-tropical jet stream where birds typically migrate, supporting the avian dispersal hypothesis.

Keywords: avian carriers, Great Salt Lake, halophiles, haloarchaea, microbial biogeography

Acknowledgements. This work was suported by the NASA Space Grant Prime Award (NNX15A124H, Sub-Award 10037896WEST), the W.M. Keck Foundation, and the Lawrence T. and Janet T. Dee Foundation.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 43)

The ecological importance of solar saltworks

Nikolaos A. Korovessis[1],✉

[1]*Hellenic Saltworks S.A., 1, Asklipiou str., 10679 Athens, Greece;*
✉***Corresponding author, E-mail: nkor@ceissa.org.***

Abstract

Solar saltworks have produced salt by solar evaporation of seawater since the dawn of human civilization. However, recognition of the unique coastal ecosystem that has progressively developed, in parallel with the sea salt production process evolution is often lacking. Current, industrial solar saltworks consist of a series of successive, interconnecting lakes where the seawater enters and its density gradually increases by solar evaporation, as it flows under controlled conditions into the lake system. Finally, a salinity vector develops throughout the lakes system, which results in salt crystallization in the later lake in the raw. Along with the salinity vector, an extremely important biological process also develops in every lake, consisting of planktonic and benthic communities of microorganisms that cover all existing domains of life, *Eukaryota*, *Bacteria* and *Archaea*. As each lake functions at steady state, a unique ecosystem is created where regular and hypersaline environments coexist. The physical and the biological process of solar saltworks interact strongly and affect both the quantity and quality of their final product. In this paper, we examine the key role of the crustacean *Artemia salina* compared to that of the protozoan *Fabrea salina*. The presence of these microorganisms decisively affects the microbial synthesis in crystallizers, which in turn determines the salt crystallization process. Current solar saltworks, apart for being profitable industries, establish high significant saline wetlands, offering their contribution in safeguarding wetland areas globally. They simply prove that development and environmental protection can indeed go hand in hand.

Keywords: *Fabrea salina*, sea salt, solar salt, solar saltworks, wetlands.

Purple Spots on ancient parchments *vs* Red Heats on present-day leathers: the unveiled secret of the salt-cured hides

Nicoletta Perini[1,✉], Fulvio Mercuri[1], Silvia Orlanducci[1], Maria Cristina Thaller[1], Alessandro Rubechini[2], Domenico Castiello[3], Valerio Talarico[3] and Luciana Migliore[1]

[1]Tor Vergata University, Via della Ricerca Scientifica, snc, 00133, Rome, Italy; [2]Vatican Secret Archives, Vatican City, Vatican City; [3]Po.Te.Co. s.c.r.l., Via San Tommaso, 119/121/123, 56029, Santa Croce sull'Arno (Pisa), Italy; ✉***Corresponding author, E-mail: niper87@gmail.com.***

Abstract

Leather products, used in many ways over centuries, can easily undergo biodeterioration. Modern hides are often damaged by red patches, known as red heats; historical parchments show similar signs of biodeterioration, the purple spots. A two-phases ecological succession explained the parchment biodeterioration process, identifying *Halobacterium salinarum* as the main culprit of purple spots, entering together with salt into the hide during the brining, in the first phase; whilst, the second phase changes with the history of each parchment (Migliore *et al.,* 2017, 2019). To evaluate if similar biodeterioration dynamics can be identified in both leather and parchment, a multidisciplinary study was performed on present-day leathers. This approach consisted of standard cultivation methods and molecular, chemical and physical updated technologies, as Next-Generation Sequencing (NGS), Raman spectroscopy and Transmitted Light Analysis (LTA). As a whole, (i) NGS demonstrated that leathers share the same triggering halophilic agents responsible for the parchment damage; (ii) Raman analyses detected bacteriorhodopsin in red patches; (iii) LTA showed that chrome tanning, conversely to parchment procedure, chemically stabilizes even the damaged areas, making leather products persistent.

Keywords: brine cured hides, *H. salinarum,* purple spots, red heat deterioration

References

Migliore, L., Thaller, M.C., Vendittozzi, G., Mejia, A.Y., Mercuri, F., Orlanducci, S., & Rubechini, A. (2017). Purple spot damage dynamics investigated by an integrated approach on a 1244 A.D. parchment roll from the Secret Vatican Archive*. Sci Rep* **7**: 9521.

Migliore, L., Perini, N., Mercuri, F., Orlanducci, S., Rubechini, A., & Thaller, M.C. (2019). Solving the jigsaw of the parchment purple spot deterioration: three documents of the XVI-XVII century validate the microbial succession model*. Sci Rep* **9**: 1623.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 45)

Interaction of uranium with halophilic microorganisms

Miriam Bader[1], Stephan Hilpmann[1], Juliet S. Swanson[2], Robin Steudtner[1], Björn Drobot[1], Matthias Schmidt[3], André Rossberg[1], Atsushi Ikeda-Ohno[1], Thorsten Stumpf[1] and Andrea Cherkouk[1,✉]

[1]Helmholtz-Zentrum Dresden-Rossendorf, Institute of Resource Ecology, Bautzner Landstrasse 400, Dresden, Germany; [2]Los Alamos National Laboratory, Repository Science and Operations, Carlsbad, New Mexico, United States of America; [3]Helmholtz Centre for Environmental Research–UFZ, Department of Isotope Biogeochemistry, ProVIS, Leipzig, Germany;
✉***Corresponding author, E-mail: a.cherkouk@hzdr.de.***

Abstract

Rock salts are considered as potential host rocks for the long-term storage of highly radioactive waste in a deep geological repository. In addition to bacteria and fungi, extremely halophilic archaea, e.g. *Halobacterium* species, are predominantly present in this habitat. For long-term risk assessment it is of high interest to study how these microorganisms can potentially interact with radionuclides if the radionuclides are released from the waste repository. Given this fact, the interactions of extremely halophilic archaea from the genus *Halobacterium* and the moderately halophilic bacterium *Brachybacterium* sp. G1 with uranium, one of the major radionuclides of concern in the geological repository of radioactive wastes, were investigated in detail in batch experiments. The archaea and the bacterium showed different association mechanisms with uranium. *Brachybacterium* sp. G1 cells sorbed uranium within a short time, whereas a much longer and a multi-stage bioassociation process, dependent on the uranium concentration, occurred with the archaea. Furthermore, a multi-spectroscopic (time-resolved laser-induced fluorescence spectroscopy and X-ray absorption spectroscopy) and -microscopic (scanning electron microscopy coupled with energy-dispersive X-ray analysis for elemental mapping) approach was used to elucidate the U(VI) bioassociation behavior. By using these spectroscopic and microscopic tools, the formation of a U(VI) phosphate mineral, such as meta-autunite, by the *Halobacterium* species was demonstrated. These findings offer new insights into the microbe-actinide interactions at highly saline conditions relevant to the disposal of nuclear waste.

Keywords: deep geological repository, halophiles, uranium.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 46)

Uncovering the microbial interactome: The social life of extremophiles

Noor-ul-Huda Ghori[1,✉], Michael Wise[2] and Andrew S. Whiteley[1]

[1]The University of Western Australia, The UWA School of Agriculture and Environment (SAgE), Perth, Australia; [2]The University of Western Australia, Department of Computer Science and Engineering, Perth, Australia;
✉Corresponding author, E-mail: noor-ul huda.ghori@research.uwa.edu.au

Abstract

Lake Magic, located on the Yilgarn Craton in southern Western Australia, is a round lake with ~1 km diameter and harbours a unique environment on Earth. It exhibits co-stressors of pH 1.6-4.5 and salinity of 32% TDS. The lake is known to have the highest concentration of dissolved Al, Fe and Si in the world. Lake Magic undergoes stages of flooding, evapo-concentration and desiccations, and hence, is a home to a diverse population of halophilic and acidophilic microorganisms. Understanding the diversity dynamics and survival strategies of extremophiles enables us to comprehend how they interact to drive key biogeochemical cycles, and has broad applications in biomining, synthetic ecology and biotechnology. Here, we used a temporal approach to understand prokaryotic and eukaryotic diversity in the lake sediment and salt mat using high throughput amplicon sequencing. To further understand the survival strategies of microbes, their microbial interactions were studied using a high throughput co-culture method. We established a bottom up approach by using bacterial species isolated from Lake Magic in a well-controlled synthetic community fed with labelled isotope substrates and analysed them through NanoSIMS. We studied the species metabolic interactions in all mono and pairwise cultures. The results indicated that the microbial diversity and composition are significantly affected by lake conditions, and species become more specialised in buffering the increased acidity in the lake as a strategy to survive. NanoSIMS analysis gave an in-depth view of carbon and nitrogen exchange between isolates, shedding light on the potential tactics to survive in this extreme environment.

Keywords: acid saline lake, amplicon sequencing, co-culture, microbial ecology, NanoSIMS.

Acknowledgements. This work was supported by an Australian government PhD scholarship.

Life in a pinch of salt

Terry J. McGenity[1,✉]

[1] University of Essex, School of Biological Sciences, Wivenhoe Park, Colchester, CO4 3SQ, UK;
✉Corresponding author, E-mail: tjmcgen@essex.ac..uk.

Abstract

Hypersaline environments approaching halite precipitation are rich in microbial biomass but poor in diversity, dominated by a range of extremely halophilic Archaea (haloarchaea) and to a lesser extent by Bacteria such as *Salinibacter ruber*. The phototrophic Eukaryote, *Dunaliella salina*, is a major primary producer in brines prior to halite precipitation. These extremely halophilic microbes, especially the haloarchaea, become trapped inside the brine inclusions of halite as it precipitates. The entombed microbes are thus protected from desiccation, UV light and the increasing salinity and chaotropicity of the remaining brine. By temporarily surviving in this microenvironment they can grow in larger bodies of brine formed when the halite dissolves. Analysis of the haloarchaea inside halite crystals from ~30 coastal solar salterns across the globe revealed three abundant species present in all crystals: *Halolamina sediminis, Halobacterium noricense* and *Halorubrum orientale* (Clark *et al.*, 2017). Laboratory experiments showed that *Halobacterium* species survive better in halite than the other microbes tested, and that co-entombment of mixed species enhances survival (Gramain *et al.*, 2011). The idea that survival inside halite may extend over geological time will be discussed.

Keywords: haloarchaea, longevity.

Acknowledgements. This work was suported by the European Union's Horizon 2020 programme: Marie Skłodowska-Curie grant 765256, SaltGiant, and COST Action CA15103, MEDSALT; and the University of Essex.

References

Clark, D. R., Mathieu, M., Mourot, L., Dufossé, L., Underwood, G. J. C, Dumbrell, A. J., & McGenity, T.J. (2017). Biogeography at the limits of life: do extremophilic microbial communities show biogeographic regionalisation? *Global Ecol Biogeogr* **26:** 1435–1446.

Gramain, A., Chong Díaz, G., Demergasso, C., Lowenstein, T.K., & McGenity, T. J. (2011). Archaeal diversity along a subterranean salt core from the Salar Grande (Chile). *Environ Microbiol* **13:** 2105–2121.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 48)

Marine-freshwater transitions require extensive changes in the proteome. What it takes to live without salt

Francisco Rodriguez-Valera[1,✉] and Pedro Cabello-Yeves[1]

[1] Evolutionary Genomics Group, Universidad Miguel Hernandez, San Juan de Alicante, Spain;
✉Corresponding author, E-mail: frvalera@umh.es.

Abstract

The comparison of microbial genomes found in either freshwater or marine habitats indicated that in some cases (*Synechococcus* and *Ca*. Pelagibacter) there were notable differences in the global isoelectric point (pI) of proteins. We have analysed global metagenomic proteomes and have added more prokaryotes to extend the pI comparison. Without exception, in a set that included archaea and multiple bacterial phyla, the proteome pI distribution varied, with more acidic values in marine and neutral/basic in freshwater microbes. Four pairs of highly related prokaryotes of marine and freshwater origin revealed marked differences manifested mostly in the residues located at the protein surface. This study has also shown that the magnitude of the change depended on protein location (secreted > cytoplasmic > transmembrane) and affected proteins encoded at both core and flexible genome. Our results point to a very extensive variation taking place in microbes when they move from marine (salt-rich) to freshwater habitats. These adaptations would require long evolutionary times to produce changes involving many genes in the core genome. They also point to significant differences in the physiology, probably at the level of membrane functioning, bioenergetics, intracellular ion concentration and pH (or all of them).

Keywords: intracellular ions, isoelectric point, proteomics.

Acknowledgements. This work was suported by Spanish Ministry of Science Grant VIREVO CGL2016-76273-P

Microbial diversity in the deep-sea anaerobic hypersaline environments with emphasis on the role of anaerobic haloarchaea in C and S cycles

Michail M. Yakimov[1,✉], Violetta La Cono[1] and Dimitry Yu. Sorokin[2,3]

[1] Institute for Biological Resources and Marine Biotechnology, IRBIM-CNR, Spianata San Raineri, 86, 98122 Messina, Italy; [2] Winogradsky Institute of Microbiology, Research Centre of Biotechnology, Russian Academy of Sciences, Moscow, Russia; [3] Department of Biotechnology, Delft University of Technology, Delft, The Netherlands; ✉***Corresponding author, E-mail: mikhail.iakimov@cnr.it.***

Abstract

Extremely halophilic archaea represented by classes *Halobacteria*, *Methanonatronarchaeia* and candidate division Nanohaloarchaea apparently originated from two distinct clades of methanogenic anaerobes. The members of class *Halobacteria* within the phylum *Euryarachaeota* are hypothesized to acquire by a massive lateral LTG event of ~1,000 eubacterial genes (30% of their genomes). As a consequence of this acquisition, most of the known *Halobacteria* species are aerobic heterotrophs inhabiting oxygenated layers of brines with very few of them possessing the ability for fermentative growth and/or anaerobic respiration. Our recent research, aimed at microbial sulfur respiration at extreme salinity in anaerobic sediments, uncovered the existence of at least two novel functional groups of strict anaerobic sulfidogenic haloarchaea. One group is using acetate as the electron donor for elemental sulfur-dependent respiration - a catabolic route overlooked previously in the whole Archaeal Kingdom. The second group uses formate and/or H_2 as the electron donor and elemental sulfur, thiosulfate or DMSO as the alternative acceptors thus, representing a first example of lithoheterotrophy in haloarchaea. The discovery of these groups of obligate anaerobic sulfur-respiring haloarchaea, widely present in anoxic hypersaline environments, including deep-sea brine lakes, showed that (i) the carbon and sulfur cycles in anoxic hypersaline ecosystems should be reconsidered and (ii) the dominant paradigm on the haloarchaeal physiology is far from completeness.

Keywords: deep-sea anoxic brine lakes, extreme halophilic euryarchaea, genome sequence, *Halobacteria*, sulphur respiration.

Acknowledgements. This work was supported by the Italian Ministry of University and Research under RITMARE Flagship Project (2012–2016) and by the “INMARE” Project (Contract H2020-BG-2014-2634486), funded by the European Union’s Horizon 2020 Research Program.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 50)

Integrating geology and microbiology to understand preservation of extremophiles in salt minerals

Kathleen C. Benison[1,✉]

[1] *West Virginia University, Department of Geology and Geography, Morgantown, West Virginia, U.S.A.;* ✉***Corresponding author, E-mail: kcbenison@mail.wvu.edu.***

Abstract

Bedded halite and gypsum form by rapid precipitation from surface lake, seawater, and spring brines, allowing entrapment of brines as primary fluid inclusions. Air and other gases, solids, and microorganisms may exist within fluid inclusions or as solid inclusions. Recent advances permit *in situ* observations of morphologies, optical characteristics, and compositional signatures of microorganisms and organic compounds in halite and gypsum from acd salt lakes (i.e., Conner & Benison, 2013; Benison & Karmanocky, 2014). Improved long-working distance microscope objectives enable magnification up to 2000 x and can be used with transmitted and UV light to image and analyze cells as small as 1-2 microns. Transmitted light microscopy has detected prokaryotic cocci and rods, *Dunaliella* algae, pennate diatoms, carotenoids and waxes in modern acid lake halite and gypsum. UV florescent response and laser Raman spectroscopy characterize organic signatures. Fieldwork, paired with geochemical and microbiological analyses, indicates host conditions for microbial communities (i.e., Zaikova *et al*., 2018). Permian halite from acid saline lake deposits contains microbial features similar to modern counterparts. Implications include new understanding of early terrestrial life and advisement for the search for life on Mars.

Keywords: extremophiles, fluid inclusions, gypsum, halite, microorganisms

Acknowledgements. This work was suported by NASA and NSF grants to KCB.

References

Benison, K.C., & Karmanocky, F.J., III (2014). Could microorganisms be preserved in Mars gypsum? Insights from terrestrial examples. *Geology* **42**(7):615-618.

Conner, A. J., & Benison, K.C. (2013). Acidophilic halophilic microorganisms in fluid inclusions in halite from Lake Magic, Western Australia. *Astrobiology* **13**(9): 850-860.

Zaikova, E., Benison, K. C., Mormile, M.R., & Johnson, S.S. (2018). Microbial communities and their predicted metabolic functions in a desiccating acid salt lake. *Extremophiles* **22**(1):367-379.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 51)

Haloviruses: the bad, the worse and the surprising

Mike Dyall-Smith[1,✉], Sen-Lin Tang[2] and Friedhelm Pfeiffer[1]

[1]*Computational Biology Group, Max-Planck-Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany;* [2]*Biodiversity Research Center, Academia Sinica, Taipei, Taiwan;*
✉***Corresponding author, E-mail: mike.dyallsmith@gmail.com.***

Abstract

Relatively few haloviruses have been isolated and their genomes sequenced, even though they are present at high levels in hypersaline waters, and are likely to be major drivers of host species evolution. We recently published the genome sequences of two haloviruses, phiH1 and ChaoS9 (Dyall-Smith *et al*., 2018, 2019), both myoviruses infecting *Halobacterium salinarum*, and both isolated following lysis events of laboratory cultures used to produce bacteriorhodopsin. While phiH caused a minor loss of production, ChaoS9 was much worse, lysing a 1 m^3 culture. The two viral genomes are remarkably similar, even though they originated from independent lysis events many years apart. Both genomes were also similar to halovirus phiCh1 (host: *Natrialba magadii*), and while the three show many patchy differences (mosaicism), they form a distinct clade by various methods of phylogenetic tree reconstruction. Surprisingly, the major capsid protein (MCP) of ChaoS9 is not similar to those of phiH1 or phiCh1, but is closely related to the MCP of HHTV-1 (host: *Haloarcula hispanica*), a siphovirus that is otherwise unrelated. In addition, a number of previously unexamined halovirus isolates, kept frozen as clarified cell lysates for many years, have been analysed by high-throughput sequencing of total DNA. This revealed the presence not only of novel haloviruses but also proviruses derived from the host cell genomes. The surprising presence of induced proviruses may be common in haloarchaea, and could potentially threaten experimental studies or large scale technical applications involving two or more species.

Keywords: haloarchaea, halobacteria, halovirus, hypersaline, provirus.

Acknowledgements. This work was suported by (a) the Max Planck Society, Germany (to D. Oesterhelt, Dept Biochemistry), and (b) BRC, Academia Sinica, Taiwan

References

Dyall-Smith, M., Pfeifer, F., Witte, A., Oesterhelt D., & Pfeiffer, F. (2018). Complete genome sequence of the model halovirus phiH1 (ΦH1). *Genes (Basel)* **9**(10): 493.

Dyall-Smith, M., Palm, P., Wanner, G., Witte, A., Oesterhelt D., & Pfeiffer, F. (2019). Halobacterium salinarum virus ChaoS9, a novel halovirus related to phiH1 and phiCh1. *Genes (Basel)* **10**(3): 194.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 52)

Structural-functional analysis of extremely hypersaline microbial mats from the Arabian Peninsula

Raeid M. M. Abed[1,✉]

[1]Sultan Qaboos University, Al-Khoud 123, P. O. Box 36, Muscat, Sultanate of Oman;
✉Corresponding author, E-mail: rabed@squ.edu.om.

Abstract

Microbial mats in most extreme settings, as found at the coastline of the subtropical-arid Arabian Peninsula, have been rarely studied. We used next-generation Illumina MiSeq sequencing, microsensors, stable isotopes and qPCR to study the diversity and carbon and nitrogen cycling in hypersaline microbial mats from Oman and UAE. Highly adapted bacterial specialists were mainly found at the most extreme, upper tidal sites and less specialized organisms with wide tolerance ranges in intermediate and lower sites of the transect. Up to 40% of the archaeal sequences represented so far unknown phyla. Rates of photosynthesis and respiration at the ambient salinities of the mats revealed a decrease from the lower to the upper tidal zone. All mats, regardless of their tidal location, exhibited a decrease in photosynthesis and respiration rates at salinities > 100‰. With respect to nitrogen cycle, these mats had potential rates of ammonia oxidation and denitrification of 0.8±0.4 and 2.0±1.0 nmol N g^{-1} h^{-1}, respectively, whereas anammox was not detectable. The N_2O production rate under anoxic conditions accounted for ca. 5% of total denitrification. Based on qPCR assays, *amoA* genes had the highest copy number while *narG* and *nirS* genes exhibited comparable estimates. Sequences of *nirS* gene were novel whereas *nirK* sequences were related to sequences from the *Rhizobiales*. Sequences of the *nosZ* gene were the most diverse and clustered with sequences from various genera. We conclude that the mats in the Arabian Peninsula have adjusted the diversity and function of their microbial communities to cope with extreme conditions.

Keywords: cyanobacterial mats, denitrification, microsensors, photosynthesis, respiration.

Acknowledgements. This work was suported by Sultan Qaboos University, Oman and the Max-Planck Institute, Germany.

“Metaviromics asks, metaviroproteomics answers”

María Dolores Ramos-Barbero[1], Joseph Christie-Oleza[2], Juan Ramón Hernández-Fernaud[2], Manuel Martínez-García[1], Fernando Santos[1,✉] and Josefa Antón[1]

[1]University of Alicante, Alicante, Spain; [2]University of Warwick, Coventry, United Kingdom;
✉***Corresponding author, E-mail: Fernando.Santos@ua.es.***

Abstract

Viruses in hypersaline environments can reach up to 10^{10} particles per ml (DiMeglio *et al*., 2016). Therefore, these systems constitute a perfect scenario for virus-host interactions studies. Despite their simplicity, viral genomes can contain a high proportion of genes with unknown functions (coding for hypothetical proteins, HPs). Based on the fact that proteins in virions are mainly structural, we have applied a protocol for the study of viral proteins directly from the environment: the metaviroproteomes. We analyzed two metaviroproteomes from two ponds of Bras del Port salterns (Spain) and developed a new pipeline for the search of viral peptides against the database of proteins (DB) constructed from their corresponding metaviromes. This procedure has allowed us to increase the percentage of peptides identification respecting to the previously reported protocol (Brum *et al*., 2016), and to associate many peptides to HPs-coding ORFs, now re-annotated as “structural genes”. Also, a fraction of peptides did not match against the DB as a consequence of the ”great metagenomics anomaly” (Ramos-Barbero *et al.*, 2019), but they did match against non-assembled reads. Finally, the microdiversity contained within the structural peptides was analyzed throughout a large set of halometaviromes as a way to explore if it can be on the basis of expanding (or maintaining) the halovirus-host ranges.

Keywords: hypothetical protein, metavirome, metaviroproteome, virus

References

Brum, J.R., Brum, J. R., Ignacio-Espinoza, J. C., Kim, E. H., Trubl, G., Jones, R. M., Roux, S., VerBerkmoes, N. C., Rich, V. I., & Sullivan, M. B. (2016). Illuminating structural proteins in viral “dark matter” with metaproteomics. *Proc Natl Acad Sci USA* **113**(1): 2436-2441.

DiMeglio, L., Santos, F., Gomariz, M., Almansa, C., López, C., Antón, J., & Nercessian, D. (2016). Seasonal dynamics of extremely halophilic microbial communities in three Argentinian salterns. *FEMS Microbiol Ecol* **92**(12): fiw184.

Ramos-Barbero, M. D., Martin-Cuadrado, A. B., Viver, T., Santos, F., Martinez-Garcia, M., & Antón, J. (2019). Recovering microbial genomes from metagenomes in hypersaline environments: The Good, the Bad and the Ugly. *Syst Appl Microbiol* **42**(1): 30-40.

Viral evolution and diversification: what have we learnt from *Salinibacter* and its viruses?

María Dolores Ramos-Barbero[1], Judith Villamor[1], Tomeu Viver[2], Fernando Santos[1], Manuel Martínez-García[1], Ramon Rosselló-Móra[2] and Josefa Antón[1,✉]

*[1]University of Alicante, Apatardo 99, 03080 Alicante, Spain; [2]Mediterranean Institute for Advanced Studies (IMEDEA), Department of Animal and Microbial Biodiversity, Marine Microbiology Group, Esporles, Spain; ✉**Corresponding author, E-mail: anton@ua. es.***

Abstract

Salinibacter ruber is frequently the most abundat bacterium in hypersaline environments but its numbers rarely reach that of the most abundant archaea. Using a combination of viral metagenomics of natural and manipulated hypersaline systems, culture and microscopy techniques, we undertook the study of *S. ruber* viruses to understand their role in controlling hosts' abundance and diversity in nature. So far, we have isolated more than 200 new „saliniviruses" representing four new families within the Caudovirales. The monitoring of their genomes in nature together with the study of *S. ruber* viruses reconstructed *in silico* shows how both mutation and island acquisition shape their evolution. Furthermore, these studies unveil how rapidly viral communities can accomodate to changes in hosts populations.

Keywords: hypersaline, metagenomic island, *Salinibacter*, salinivirus, viral metagenome

Acknowledgements. This work was funded by the EU grant LEIT-BIO-2015-685474, Metafluidics, (to JA) and the grants CLG2015 66686-C3-1 and 3 (to JA and RRM, respectively) of the Spanish Ministry of Economy and Competitiveness, which included FEDER funds.

References

Ramos-Barbero, M.D. (2019). Ecogenomics of viruses from hypersaline environments. PhD. Thesis, University of Alicante.

Villamor, J., Ramos-Barbero, M.D., González-Torres, P., Gabaldón, T., Rosselló-Móra, R., Meseguer, I., Martínez-García, M., Santos, F., & Antón J. (2018). Characterization of ecologically diverse viruses infecting co-occurring strains of cosmopolitan hyperhalophilic Bacteroidetes. *ISME J* **12**(2): 424-437.

Bioenergetics of haloalkaliphiles

Maria S. Muntyan[1, ✉], Anssi M. Malinen[2], Dimitry Y. Sorokin[3] and Vladimir P. Skulachev[1]

[1]Lomonosov Moscow State University, Belozersky Institute of Physico-Chemical Biology, Bioenergetics, Moscow, Russia; [2]University of Turku, Biochemistry, Turku, Finland; [3]Delft University of Technology, Biotechnology, Delft, The Netherlands;
✉ ***Corresponding author, E-mail: muntyan@genebee.msu.ru.***

Abstract

The research aims at finding out the distinctive lines of energetics in natronophilic microorganisms started to be described (Banciu & Muntyan, 2015). Among used methods were analyses of respiratory characteristics, electrical potential generation, pH changes in cells/vesicles suspension upon light/oxygen impulses, effects of ionophores and uncouplers, visualization of sodium transport using radioisotope ^{22}Na, phylogenetics. Summary of results: So far, a novel type of primary energy transformer, Na^+-motive cytochrome oxidase, which has been proven to operate in natronophilic strains of the genus *Thioalkalivibrio* (Muntyan *et al.*, 2015), has been discovered, and then found in several other extremophiles. It has been demonstrated that in these same strains, cell motility is provided by Na^+-motive flagella. In addition, it was shown that the rhodopsin-like pigment, proteorhodopsin, in the new natronophilic strain of a novel deep-lineage of the phylum Balneolaeota, *Cyclonatronum proteinivorum*, pumps Na^+ from cells (Sorokin *et al.,* 2018). The screening of Na^+-motive energy mechanisms revealed the sodium energy cycle, consisting of (i) primary mechanisms for generating Na^+-potential and (ii) Na^+-potential consumers, represented by flagella and FoF1-ATPase. Conclusion: Along the way, we first discovered that several species of bacteria simultaneously have in their genomes: (i) oxygen-consuming generators of Na^+-potential (Na^+-pumping *cbb*$_3$ oxidases) and (ii) consumers of Na^+-potential such as Na^+-ATPase of F0F1-type and flagella. Thus, for the first time, it became possible to establish the presence of a complete Na^+-cycle in energetics of oxygen-respiring bacteria.

Keywords: alkaliphiles, Na^+-motive enzymes, soda lakes.

Acknowledgements: Russian Foundation for Basic Research-grant 17-04-02173.

References

Banciu, H.L., & Muntyan, M.S. (2015). Adaptive strategies in the double-extremophilic prokaryotes inhabiting soda lakes. *Curr Opin Microbiol* **25**: 73-79.

Muntyan, M.S., Cherepanov, D.A., Malinen, A.M., Bloch, D.A., Sorokin, D.Y., Severina, I., Ivashina, T.V., Lahti, R., Muyzer, G., & Skulachev, V. P. (2015). Cytochrome cbb_3 of *Thioalkalivibrio* is a Na^+-pumping cytochrome oxidase. *Proc Natl Acad Sci USA* **112**(25): 7695-7700.

Sorokin, D.Y., Muntyan, M.S., Toshchakov, S.V., Korzhenkov, A., & Kublanov, I.V. (2018). Phenotypic and genomic properties of a novel deep-lineage haloalkaliphilic member of the phylum Balneolaeota from soda lakes possessing Na^+-translocating proteorhodopsin. *Front Microbiol* **9**: 2672.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 57-58)

The extremolytes ectoine and hydroxyectoine: import, synthesis and genetic regulation in response to osmotic stress

Erhard Bremer[1,✉]

[1] *Philipps-Universität Marburg, Laboratory for Microbiology, Department of Biology, Karl-von-Frisch Straße 8, 35043 Marburg, Germany;*
✉***Corresponding author, E-mail: bremer@staff.uni-marburg.de.***

Abstract

When exposed to high osmolarity/salinity environments, many *Bacteria* and *Archaea* counteract the ensuing dehydration of the cytoplasm and drop in vital turgor though the accumulation of a selected class of organic osmolytes, the compatible solutes. These compounds are highly compliant with cellular physiology. In addition to their crucial role in cellular osmotic adjustment, they also serve as chemical chaperones by promoting the functionality of cellular components, macromolecular complexes, and even of entire cells from various types of insults. Ectoine and its derivative hydroxyectoine are prominent members of the compatible solutes. Their biosynthetic genes (*ectABC/D*) are widely found in members of the *Bacteria* (Czech *et al*., 2018), occur only rarely in *Archaea* (Widderich *et al*., 2016) and, surprisingly, have now also been detected in a few halophilic ciliates. It seems that the *ect* genes-containing *Archaea* and ciliates have acquired the ability to produce ectoines via horizontal gene transfer events (Widderich *et al*., 2016; Czech & Bremer, 2018). Ectoines can be amassed by osmotically stressed cells through import and synthesis. I will discuss various types of ectoine/hydroxyectoine importers with special emphasis on the substrate-binding-proteins of ABC- and TRAP-type transporters as their crystal structures revealed the molecular determinants of how compounds that are otherwise preferentially excluded from protein surfaces can nevertheless be bound with high affinity and specificity (Hanekop *et al*., 2007; Lecher *et al*., 2009). In addition, I will present an overview of the biochemistry and structural biology of the ectoine/hydroxyectoine biosynthetic enzymes (Höppner *et al*., 2014; Czech *et al*., 2019), and discuss the osmostress-responsive expression of the *ect* biosynthetic genes (Czech *et al*., 2018).

Keywords: ectoine, gene regulation, hydroxyectoine, osmotic stress, synthesis, transport.

Acknowledgements. This work was supported by the German Research Council (DFG) through the Collaborative Research Center 987 (project number: 192445154-SFB 987).

References

Czech, L., & Bremer, E. (2018). With a pinch of extra salt—Did predatory protists steal genes from their food?. *PLoS Biol* **16**(2): e2005163.

Czech, L., Poehl, S., Hub, P., Stoeveken, N., & Bremer, E. (2018). Tinkering with osmotically controlled transcription allows enhanced production and excretion of ectoine and hydroxyectoine from a microbial cell factory. *Appl Environ Microbiol* **84**(2): e01772-17.

Czech, L., Hermann, L., Stöveken, N., Richter, A., Höppner, A., Smits, S., Heider, J., & Bremer, E. (2018). Role of the extremolytes ectoine and hydroxyectoine as stress protectants and nutrients: genetics, phylogenomics, biochemistry, and structural analysis. *Genes* **9**(4): 177.

Czech, L., Höppner, A., Kobus, S., Seubert, A., Riclea, R., Dickschat, J. S., Heider, J., Smits, S. H., & Bremer, E. (2019). Illuminating the catalytic core of ectoine synthase through structural and biochemical analysis. *Sci Rep* **9**(1): 364.

Hanekop, N., Höing, M., Sohn-Bösser, L., Jebbar, M., Schmitt, L., & Bremer, E. (2007). Crystal structure of the ligand-binding protein EhuB from *Sinorhizobium meliloti* reveals substrate recognition of the compatible solutes ectoine and hydroxyectoine. *J Mol Biol* **374**(5): 1237-1250.

Höppner, A., Widderich, N., Lenders, M., Bremer, E., & Smits, S. H. (2014). Crystal structure of the ectoine hydroxylase, a snapshot of the active site. *J Biol Chem* **289**(43): 29570-29583.

Lecher, J., Pittelkow, M., Zobel, S., Bursy, J., Bönig, T., Smits, S. H., Schmitt, L., & Bremer, E. (2009). The crystal structure of UehA in complex with ectoine—a comparison with other TRAP-T binding proteins. *J Mol Biol* **389**(1): 58-73.

Widderich, N., Czech, L., Elling, F. J., Könneke, M., Stöveken, N., Pittelkow, M., Riclea, R., Dickschat, J. S., Heider, J., & Bremer, E. (2016). Strangers in the archaeal world: osmostress-responsive biosynthesis of ectoine and hydroxyectoine by the marine thaumarchaeon *Nitrosopumilus maritimus*. *Environ Microbiol* **18**(4): 1227-1248.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 59)

Effect of ectoine on DNA: Mechanisms of interaction and protection

Hans Jörg Kunte[1,✉] and Susan Meyer[1]

*[1]Bundesanstalt für Materialforsschung und -prüfung (BAM), Unter den Eichen 87, Berlin, Germany; ✉**Corresponding author, E-mail: hans-joerg.kunte@bam.de.***

Abstract

The compatible solute ectoine is a versatile protectant synthesized by many prokaryotes. It is used as an osmolyte helping microorganisms to maintain an osmotic equilibrium. In addition, ectoine acts as a stabilizer and protects proteins, membranes and whole cells against detrimental effects such as freezing and thawing, drying and high temperatures. Its protective effect is explained by the *preferential exclusion* model, which postulates that ectoine does not directly interact with biomolecules but is excluded from their surface. Interestingly, details on the interaction of ectoine with DNA are still unknown. Therefore, we studied the influence of ectoine on DNA and the mechanisms by which ectoine protects DNA against ionizing radiation. To emulate biological conditions, we used a sample holder comprising a silicon chip with a Si_3N_4 membrane, which allows for electron irradiation of DNA in aqueous solution. Analysis by atomic force microscopy revealed that without ectoine, DNA was damaged by irradiation with a dose of 1,7 +/-0,3 Gy. With ectoine, DNA remained undamaged, even after irradiation with 15 Gy. Simulations with dsDNA and ectoine in water revealed a *preferential binding* of the zwitterionic ectoine to the negatively charged DNA. According to the simulations, binding of ectoine will destabilize dsDNA. Destabilizing is probably caused by the transition of B-DNA to A-DNA and will reduce the DNA melting temperature, which was experimentally proven. The preferential binding provides a stable ectoine shell around DNA, which allows ectoine to reduce OH-radicals and electrons near the DNA and thereby mitigating the damaging effect of ionizing radiation.

Keywords: ectoine, ionizing radiation, preferential binding.

References

Schröter, M. A., Meyer, S., Hahn, M. B., Solomun, T., Sturm, H., & Kunte, H. J. (2017). Ectoine protects DNA from damage by ionizing radiation. *Sci Rep* **7**(1): 15272.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 60-61)

Synthesis, uptake and excretion of ectoines in the alpha-proteobacteria *Hyphomonas neptunium* and *Novosphingobium* sp. LH128

Laura Czech[1,✉], Sander Smits[2] and Erhard Bremer[1,✉]

[1]*Philipps-Universität Marburg, Laboratory for Microbiology, Department of Biology, Karl-von-Frisch Straße 8, 35043 Marburg, Germany;* [2]*Center for Structural Studies, Heinrich-Heine-University Düsseldorf, D-40225 Düsseldorf, Germany.* ✉***Corresponding authors, E-mail: czechla@staff.uni-marburg.de, bremer@staff.uni-marburg.de***

Abstract

A broad range of *Bacteria* either synthesizes the compatible solutes ectoine and hydroxyectoine or employ specialized transporters for their uptake to ameliorate high osmolarity stress. Through bioinformatics of all sequenced microbial genomes, more than 4000 putative ectoine producing bacteria were recently identified (Czech *et al.*, 2018). A subset of these putative ectoine producers encodes different types of transporters in the direct gene neighborhood of the core biosynthetic genes (*ectABCD)* for ectoines. Using *Hyphomonas neptunium* and *Novosphingobium* sp. LH128 as model organisms, we analyzed the synthesis, uptake and excretion of ectoines as these alphaproteobacteria possess novel types of *ect* gene clusters that harbor a different set of transporter genes, and in the case of *Novosphingobium*, also a *mscS*-like mechanosensitive channel gene. Here we show that *H. neptunium* harbors a novel sodium solute superfamily importer (EctI) exhibiting a substrate spectrum for a variety of compatible solutes. *Novosphingobium* sp. LH128, a pure hydroxyectoine producer, employs a major facilitator superfamily importer (EctU) with substrate-specificity for ectoine and hydroxyectoine. We were able to study the putative substrate-binding site of EctI and EctU for the substrates glycine betaine, ectoine and hydroxyectoine by modeling and docking studies, and through site-directed mutagenesis. In case of *Novosphingobium* sp. LH128, we showed that a knock-out mutant of EctU resulted in an hydroxyectoine secreting strain, highlighting the role of this substrate-specific importer in the recycling of the compatible solute hydroxyectoine. Furthermore, we reveal here for the first time that EctE from *Novosphingobium* sp. LH128 probably serves as a specific exporter for ectoines.

Keywords: alpha-proteobacteria, ectoine, export, salt stress, transport.

Acknowledgements: This work was supported by the German Research Council (DFG) through the CRC987, and by the International Max Planck Research School (IMPRS-Mic) through a PhD fellowship to LC.

References

Czech, L., Hermann, L., Stöveken, N., Richter, A.A., Höppner, A., Smits, S.H.J., Heider, J., & Bremer, E. (2018). Role of the extremolytes ectoine and hydroxyectoine as stress protectants and nutrients: Genetics, phylogenomics, biochemistry, and structural analysis. *Genes* **9**(4): 177.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 62)

C-terminal lipid-anchoring of *Haloferax volcanii* surface proteins

Mechthild Pohlschröder[1,✉], Anthony DiLucido [1], Friedhelm Pfeiffer[1] and F. Mohd Abdul Halim[1]

[1] *University of Pennsylvania, Department of Biology, Philadelphia, PA 19104;USA;*
✉***Corresponding author, E-mail: pohlschr@sas.upenn.edu.***

Abstract

The microbial cell surface proteins play various critical roles such as mediating the intercellular communications, nutrient uptake, surface adhesion, motility and providing cell shape. Many surface proteins are anchored via insertion of a transmembrane (TM) domain in the cytoplasmic membrane or are covalently-linked with a lipid moiety to N-terminally anchor it to the membrane. Recently, a novel anchoring mechanism in which the proteins are C-terminally processed, and covalently-lipid anchored at its C-terminus via an enzyme known as archaeosortase (ArtA) has been identified. Target proteins that are recognized and processed by ArtA carry a distinct C-terminal tripartite structure consisting of a conserved PGF motif, followed by a hydrophobic domain and positively charged residues. Molecular biological, biochemical and microscopic analyses of the model archaeon *Haloferax volcanii* confirmed the importance of the conserved PGF domain for ArtA-dependent processing, identified ArtA-dependent processing of diverse Tat as well as Sec substrates, including the S-layer glycoprotein, and revealed the active site of the ArtA peptidase. Moreover, two enzymes involved in archaetidylethanolamine biosynthesis are critical for processing and lipid anchoring of ArtA substrates. Since archaeosortases, and their bacterial homologs, the exosortases, which to date have only been studied *in silico*, are found in a broad array of archaea and bacteria, our findings have important implications for cell surface biogenesis in a wide variety of prokaryotes.

Keywords: archaeosortase, cell shape, cell surface anchoring, motility, S-layer glycoprotein.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 63)

Comparative analysis of transcription regulatory networks across the halophile phylogeny

Amy K. Schmid[1,✉]

[1] Department of Biology, Duke University, Durham, NC 27708, USA;
✉***Corresponding author, E-mail: amy.schmid@duke.edu.***

Abstract

Relative to the other domains of life, few transcription factors and the genes they control in response to stress have been functionally characterized in *Archaea*. To understand these mechanisms, we use a systems biology approach that integrates genome-wide datasets and quantitative phenotype data into predictive computational models. Such an approach characterizes the function of key global regulators and reveals how transcription factors work together to coordinate stress responses in *Halobacterium salinarum*. For example, the activity of stress-resistance and growth-response transcriptional control networks were strongly anticorrelated, suggesting a lifestyle trade-off. Here we describe the underlying transcriptional mechanisms regulating each network with a focus on the regulation of metabolism, oxidative stress, and cell division. Comparison of these transcription networks to homologous systems in other halophilic species reveals surprising functional divergence among closely related species, but conserved regulatory network architecture and dynamics with distant relatives.

Keywords: Archaea, *Halobacterium salinarum*, stress response, transcription factors.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 64)

Quorum sensing: signaling from haloarchaea to bacteria

Thomas Thompson[1], Alessandro Busetti[1], Stephen Kelly[1],
Victor Fuentes[2], Julianne Megaw[1] and Brendan Gilmore[1,✉]

[1]School of Pharmacy, Queen's University Belfast, Belfast, Northern Ireland; [2]Centro de Investigaciones Biologicas, Madrid, Spain;
✉Corresponding author, E-mail: b.gilmore@qub.ac.uk.

Abstract

Cell-cell communication through the production of autoinducer molecules has been widely studied in bacteria and found to play a pivotal role in biofilm formation and gene regulation. Quorum Sensing (QS) within the domain Archaea is understudied compared with their bacterial counterparts. The aim of this study was to determine whether haloarchaea are capable of cross-kingdom signalling through the production of QS inducing and QS inhibitory compounds. The crude extract of different haloarchaea was screened for QS induction using the AHL reporter strains *Agrobacterium tumefaciens* ATCC BAA-2240, *Chromobacterium violaceum* CV026, and the *Escherichia coli* luminescence reporters JM109 pSB536, pSB401, and psB1142. The effect on virulence factor production was assessed using the *Pseudomonas aeruginosa* mutant strain PAO-MW1. Preliminary results revealed that different genera were capable of eliciting a QS response in the *A. tumefaciens* bio-reporter. Initial characterisation of the signaling molcules using LC-MS (QTOF) analysis, TLC-overlays, and other biochemical tests, suggested the possible production of butyryl homoserine lactones or homologs from a *Halorubrum* isolate. Importantly, this extract was able to restore the production of virulence factors, pyocyanin and pyoverdine, in PAO-MW1 emphasising the capacity of an archaeal autoinducer to be sensed by bacteria. Our findings raise new questions concerning the evolution and role of QS-systems in bacteria and archaea, on the evolutionary relationship that exists between these microorganisms and on the ability of the halophilic members of these two separate domains of the tree of life to interact.

Keywords: AHLs, haloarchaea, *Halorubrum*, quorum sensing, signaling

Acknowledgements. This work was suported by Department for the Economy (DfE) NI.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 65)

Molecular mechanisms behind halotolerance of proteins

Hosein Geraili[1] and Ana Vila Verde[1,✉]

[1]Max Plank Institute of Colloids and Interfaces, Am Mühlenberg 1 OT Golm, 14476 Potsdam, Germany; ✉***Corresponding author, E-mail: ana.vilaverde@mpikg.mpg.de***

Abstract

The cytoplasmic proteins of some halophilic organisms – those with high cytoplasmic KCl concentration – have an unusually large number of acidic amino acids at their surface. This feature is thought to enable the proteins to function under those conditions, but how is yet unclear. One possible explanation is that acidic amino acids are necessary to maintain the hydration levels of the protein surface in the presence of high concentrations of K^+ and Cl^- ions. We investigate this hypothesis using molecular dynamics simulations and models we optimized to capture the balance of interactions between acidic amino acids, water and K^+. We simulate multiple halophilic proteins at both high (2 mol/kg) and low (0.15 mol/kg) KCl molality. For comparison, we simulate also proteins from mesophilic organisms and without unusually high fraction of acidic amino acids. We quantify the composition of the solvent as a function of the distance to the protein surface, and the dynamics of the first solvation layer via the mean square displacement of its components. Results for an initial subset of proteins suggest that the first solvation layer has the same water concentration as the bulk solution under all conditions tested, and that K^+ ions form preferentially solvent-shared ion pairs with the carboxylate groups in the acidic side chains. Water and ion dynamics are similar between halophiles and non-halophiles, and between high and low KCl concentrations, with diffusion coefficients differing by less than a factor of 3. Our preliminary results thus suggest that halophiles and mesophiles are similarly solvated, i.e., large numbers of acidic amino acids appear not to be necessary to maintain protein hydration at high KCl concentration. Simulations for a larger set of proteins are on-going. Conclusions based on the full set of proteins will be discussed at this meeting.

Keywords: acidic amino acids, composition of hydration layer, free energy perturbation, halophilic proteins, molecular dynamics simulations.

Acknowledgements. This work was suported by the Deutsche Forschungsgemeinschaft (proj. *Molecular mechanisms behind protein halotolerance)*

CRISPR-Cas systems in haloarchaea more than just antiviral defense?

Israela Turgeman-Grott[1], Uri Neri[1], Matthew Ouellette[2],
R. Thane Papke[2] and Uri Gophna[1,✉]

*[1]School of Molecular and Cell Biology and Biotechnology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel; [2]Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269, USA; ✉**Corresponding author, E-mail: urigo@tau.ac.il.***

Abstract

Many archaea possess spacers that match chromosomal genes of related species, including those encoding core housekeeping genes. By sequencing genomes of environmental archaea isolated from a single site, we demonstrate that inter-species spacers are common. We then showed experimentally, by mating *Haloferax volcanii* and *Haloferax mediterranei*, that spacers are indeed acquired chromosome-wide, although a preference for integrated mobile elements and nearby regions of the chromosome exists. Engineering the chromosome of one species to be targeted by the other's CRISPR–Cas reduces gene exchange between them substantially. Thus, spacers acquired during inter-species mating could limit future gene transfer, resulting in a role for CRISPR–Cas systems in microbial speciation. More recently we have identified a virus that chronically infects one of our natural *Haloferax* isolates and can also integrate into its genome. Exposure to this virus elicited strong and specific spacer acquisition by the *H. volcanii* lab strain, that surprisingly Could not be stably infected by that virus. This raised the question why the virus' original host that appears to have a functional CRISPR-Cas system did not acquire spacers from a virus that chronically infects it. We addressed this by an "active immunization" approach in which provirus-containing cells were exposed to mature virus particles.

Keywords: CRISPR-Cas, halophilic archaea, horizontal gene transfer, lateral gene transfer, viruses.

Acknowledgements. This work was suported by grants to U.G. and R.T.P. from the BSF grant to U.G. (2016671) and the NSF grant (NSF/MCB 1716046), and an ERC grant to U.G.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 67)

Evolution of chromosome architecture in *Haloferax volcanii*

Darya Ausiannikava[1], Hannah Marriott[1] and Thorsten Allers[1,✉]

[1]*School of Life Sciences, University of Nottingham, Nottingham NG7 2UH, UK;*
✉***Corresponding author, E-mail: thorsten.allers@nottingham.ac.uk***

Abstract

DNA replication is initiated at chromosomal sites called origins. Replication origins and the initiator proteins that bind them are assumed to be essential, but we have shown that in *Haloferax volcanii*, life without origins is not only possible but efficient. The DNA replication enzymes found in archaea and eukaryotes differ fundamentally from those in bacteria, but the key enzyme for homologous recombination –RecA in bacteria, RadA in archaea and Rad51 in eukaryotes – is conserved in all domains. If homologous recombination is an ancestral process that predates the split between bacteria, archaea and eukaryotes, and the evolution of their different machineries for DNA replication, could it have been used to initiate replication in the last common ancestor? We have shown that in *Haloferax volcanii*, deletion of all chromosomal origins leads to the initiation of all DNA replication by homologous recombination. Similar results have been obtained with deletion mutants lacking Orc1/Cdc6 replication initiator proteins, which are required for origin firing. Surprisingly, this leads to accelerated growth with no obvious defects, whereas deletion of origins (or initiator protein genes) in yeast or *Escherichia coli* leads to severe growth impairment. If homologous recombination alone can efficiently initiate the replication of an entire cellular genome, what purpose do replication origins serve and why they have evolved?

Keywords: DNA replication; *Haloferax volcanii*; homologous recombination; replication origin.

Acknowledgements. This work was suported by the Biotechnology and Biological Sciences Research Council (BBSRC).

References

Ausiannikava, D., Mitchell, L., Marriott, H., Smith, V., Hawkins, M., Makarova, K. S., Koonin E., Nieduszynski C., & Allers, T. (2018). Evolution of genome architecture in Archaea: spontaneous generation of a new chromosome in *Haloferax volcanii*. *Mol Biol Evol* **35**(8): 1855-1868.

Hawkins, M., Malla, S., Blythe, M. J., Nieduszynski, C. A., & Allers, T. (2013). Accelerated growth in the absence of DNA replication origins. *Nature* **503**(7477): 544-547.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 68)

Polyploidy in halophilic Archaea: regulation, evolutionary advantages, and gene conversion

Jörg Soppa[1,✉]

[1]*Goethe-University, Institute for Molecular Biosciences, Max-von-Laue-Str. 9, D-60438 Frankfurt, Germany;* ✉***Corresponding author, E-mail: Soppa@bio.uni-frankfurt.de***

Abstract

Halophilic archaea are typically polyploid and contain more than 20 copies of the major chromosome. The chromosome copy number is regulated within the growth curve and in response to environmental conditions. *Haloferax volcanii* encodes 14 paralogs of a protein that is called Origin Recognition Complex (ORC) protein. A deletion analysis of *orc* genes revealed that three genes are essential and that all ORC proteins are involved in genome copy number regulation. Origins 1 and 2 of the major chromosome have been analyzed and showed very different strengths and regulatory features. Various potential evolutionary advantages for polyploidy in haloarchaea exist, and several advantages have been addressed experimentally. Examples are a high resistance against conditions that induce DNA double strand breaks (e.g. desiccation) and the usage of genomic DNA as a phosphate storage polymer. It has been hypothesized that the function of DNA as a phosphate storage polymer might have predated the function of DNA as genetic material in evolution. It was also suggested that polyploidy might explain the survival of haloarchaea in salt deposits over geological times, and indeed new isolates from an ancient salt deposit were found to be polyploid. At least under laboratory conditions heterozygous cells can easily be selected, which contain more than one type of chromosome. In the absence of selection it has been shown that intermolecular gene conversion leads to the equalization of genome copies. Gene conversion explains how haloarchaea can escape "Muller's ratchet" and why homozygous mutants can easily be isolated or constructed.

Keywords: double strand break repair, gene conversion, *Haloferax volcanii*, origin recognition proteins, polyploidy.

Acknowledgements. This work was suported by the German Research Coucil (Deutsche Forschungsgemeinschaft) through grants So264/16 and So264/24.

References

Ludt, K., & Soppa, J. (2018). Influence of origin recognition complex proteins on the copy numbers of three chromosomes in *Haloferax volcanii*. *J Bacteriol* **200**(17): e00161-18.

Zerulla, K., & Soppa, J. (2014). Polyploidy in haloarchaea: advantages for growth and survival. *Front Microbiol* **5**:274.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 69-70)

Saturated salt is not a limit for life on Earth

John E. Hallsworth[1,✉]

[1]*Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, 19 Chlorine Gardens, Belfast, BT9 5DL, Northern Ireland,* ✉***Corresponding author, E-mail: j.hallsworth@qub.ac.uk.***

Abstract

Some extreme halophiles exhibit optimal rates of growth and metabolism at saturated NaCl (approx. 5 M; 0.755 water activity) (Stevenson *et al*., 2015; Lee *et al*., 2018). Some lower water-activity brines (acid brines, and those dominated by MgCl2 or CaCl2) are too stressful to permit metabolism (Hallsworth *et al*., 2007; Yakimov *et al.,* 2015; La Cono *et al.,* 2019; Blain, in preparation). Whereas it can be argued that microbial cells are perpetually stressed, and that stress enhances their vitality (Hallsworth, 2018), combinations of low water-activity+osmotic stress+chaotropicity, low pH, and/or high ionic strength can prevent all life processes and cause cell-system failure (Hallsworth *et al*., 2007; Blain, 2019, Alves *et al*., 2015; Fox-Powell *et al*., 2016). The question remains, what is the ultimate value at which the most-halotolerant microbe is inhibited by water activity *per se*. We aimed to investigate whether brines of <0.755 water activity can permit cellular function and found that kosmotropic substances, when incorporated into to chaotropic brines, enable proliferation of halophiles between 0.748 and 0.635 (Stevenson *et al.,* 2015; Hallsworth *et al*., 2007; Yakimov *et al*., 2015; Stevenson *et al*., 2015). Compatible solutes, essential for salt tolerance, are either chaotropic (glycerol, fructose) or kosmotropic (K^+, proline, ectoine, mannitol, trehalose) (Chin et al., 2010; Cray et al., 2013; Stevenson et al., 2017). Using the fungal xerophile/halophile *Aspergillus penicillioides* (JH06THJ; syn. FRR 6206 Williams & Hallsworth, 2009; Hallsworth, 2019), we assayed germination of conidia that had accumulated glycerol on an assay-medium supplemented with NaCl+glycerol. Differentiation, germination and cell division were observed at 0.585 water activity (Stevenson *et al.,* 2017); equivalent to a theoretical concentration of 8 M NaCl (Lee *et al*., 2018). The finite solubility of this salt may have stabilised the genetic composition of halophile populations and limited the action of natural selection in driving their evolution towards greater xerophilicity (Lee *et al*., 2018). We conclude that saturated NaCl lies only part way along the thermodynamic scale for microbial life, and does not represent a limit for life.

Keywords: astrobiology; halophile ecology; limits-for-life; osmotic stress; water activity.

References

de Lima Alves, F., Stevenson, A., Baxter, E., Gillion, J. L., Hejazi, F., Hayes, S., Morrison, I. E. G., Prior, B. A., McGenity, T. J., Rangel, D. E. N., Magan, N., Timmis, K. N., Hallsworth, J. E. (2015). Concomitant osmotic and chaotropicity-induced stresses in *Aspergillus wentii*: compatible solutes determine the biotic window. *Curr Genet* **61**: 457–477.

Blain, D., O'Neill W.K., Edwards, J., Benison, K. C., and Hallsworth, J.E. (2019, in-prep).

Chin, J. P., Megaw, J., Magill, C. L., Nowotarski, K., Williams, J. P., Bhaganna, P., et al. (2010). Solutes determine the temperature windows for microbial survival and growth. *Proc Natl Acad Sci USA* **107**: 7835-7840.

Cray, J. A., Bell, A. N. W., Bhaganna, P., Mswaka, A. Y., Timson, D. J., & Hallsworth, J. E. (2013). The biology of habitat dominance; can microbes behave as weeds? *Microb Biotechnol* **6**: 453-492.

Fox-Powell, M.G., Hallsworth, J. E., Cousins, C. R. and Cockell, C. S. (2016). Ionic strength is a barrier to the habitability of Mars. *Astrobiol* **16**: 427-442.

Hallsworth, J. E. (2018). Stress-free microbes lack vitality. *Fungal Biol* **122**: 379-385.

Hallsworth, J. E. (2019). Wooden owl that redefines Earth’s biosphere may yet catapult a fungus into space. *Environ Microbiol* doi.org/10.1111/1462-2920.14510 (in press).

Hallsworth, J. E., Timmis, K. N., McGenity, T. J., Yakimov, M. M., Golyshin, P. N., Gillion, J. *et al.* (2007). Limits of life in $MgCl_2$-containing environments: chaotropicity defines the window. *Environ Microbiol* **9**: 801-813.

La Cono, V., Bortoluzzi, G., Messina, E., La Spada, G., Smedile, F., Giuliano, L., Borghini, M., *et al*. (2019). The discovery of Lake Hephaestus, the youngest athalassohaline deep-sea formation on Earth. *Sci Rep* **9**: 1679.

Lee, C. J. D., McMullan, P. E., O’Kane, C. J., Stevenson, A., Santos, I. C., Ghosh, R. C., *et al.* (2018). NaCl-saturated brines are thermodynamically moderate, rather than extreme, microbial habitats. *FEMS Microbiol Rev* **42**: 672-693.

Stevenson, A., Burkhardt, J., Cockell, C.S., Cray, J.A., Dijksterhuis, J., Fox-Powell, M., *et al.* (2015). Multiplication of microbes below 0.690 water activity: implications for terrestrial and extraterrestrial life. *Environ Microbiol* **2**: 257–277.

Stevenson, A., Cray, J. A., Williams, J. P., Santos, R., Sahay, R., Neuenkirchen, N., McClure, C. D., Grant, I. R., *et al*. (2015). Is there a common water-activity limit for the three domains of life? *ISME J* **2**: 257-277.

Stevenson, A., Hamill, P. G., Medina, Á., Kminek, G., Rummel, J. D., Dijksterhuis, J., *et al*. (2017). Glycerol enhances fungal germination at the water-activity limit for life. *Environ Microbiol* **19**: 947-967.

Stevenson, A., Hamill, P. G., O’Kane, C. J., Kminek, G., Rummel, J. D., Voytek, M. A., *et al*. (2017). *Aspergillus penicillioides* differentiation and cell division at 0.585 water activity. *Environ Microbiol* **19**: 687-697.

Williams, J. P. and Hallsworth, J. E. (2009). Limits of Life in hostile environments: no barriers to biosphere function? *Environ Microbiol* **11**: 3292-3308.

Yakimov, M. M., La Cono, V., Spada, G. L., Bortoluzzi, G., Messina, E., Smedile, F., *et al*. (2015). Microbial community of the deep-sea brine Lake Kryos seawater-brine interface is active below the chaotropicity limit of life as revealed by recovery of mRNA. *Environ Microbiol* **17**: 364-382.

Mineral preservation and photoprotective attributes of halophilic bacteria and archaea in modern and Permian lakebed environments

Scott M. Perl[1,2,✉], Bonnie K. Baxter[3] and Aaron J. Celestian[2]

[1]California Institute of Technology / NASA Jet Propulsion Laboratory, 4800 Oak Grove Drive, Pasadena, CA 91109, United States; [2]Mineral Sciences, Los Angeles Natural History Museum, 900 Exposition Blvd, Los Angeles, CA 90007, United States; [3]Great Salt Lake Institute, Westminster College, 1840 South 1300 East, Salt Lake City, UT 84105;
✉Corresponding author, E-mail: scott.m.perl@jpl.nasa.gov

Abstract

Biogenic and inorganic matter can be preserved within inclusions during evaporite mineralization from intermittent hypersaline lakebed systems. The amount of included material within the crystalline precipitates depends on source volumes and time-scales of lakebed formation events (Baxter *et al.,* 2007; Cockell *et al.,* 2018) where photoprotective responses from halophilic organisms are present. We have compared these features against different climate and seasonal settings where solar flux is near constant versus seasonal or removed (buried) completly. These photoprotective features (e.g. carotenoids), can be quantified alongside minerals that assist in the preservation process of both organic byproducts (lipids) and microbial communities (Perl *et al.*, 2019) that have different preservation potentials due to mineral components. The purpose of this presentation is to review the modern hypersaline sites and compare preservation metrics of biogenic features in varied environments and to give insight to upcoming Martian in-situ and future Mars Sample Return analyses.

Keywords: clays, evaporites, halophiles, Mars, photoprotection, salt.

Acknowledgements. This work was suported by the JPL/Caltech Presidents and Directors Research Fund, and the JPL Research & Technology Development Fund.

References

Perl, S. M., *et al.* (2019, in-prep). Evaporitic preservation of modern carotenoid biomarkers and halophilic life in Martian analogue hypersaline environments.

Baxter, B. K., *et al.* (2007). Evidence for desiccation tolerance and ultraviolet radiation resistance. In: Instruments, Methods, and Missions for Astrobiology X, 6694:669415. SPIE, Bellingham, WA.

Cockell, C. S., Holt, J., Campbell, J., Groseman, H., Josset, J. L., *et al.* (2018). Subsurface scientific exploration of extraterrestrial environments (MINAR 5) *Int J Astrobiol* **18**(2): 157-182.

Raman spectroscopy to detect biomarkers of halophiles in salts of evaporites: from natural colonisations to synthetic inclusions

Jan Jehlička[1,✉], Adam Culka[1] and Aharon Oren[2]

[1]Institute of Geochemistry, Mineralogy and Mineral Resources, Faculty of Science, Charles University, 12843 Prague, Czech Republic; [2]Institute of Life Sciences, The Hebrew University of Jerusalem, 9190401 Jerusalem, Israel; ✉**Corresponding author, E-mail: jehlicka@natur.cuni.cz**

Abstract

Suitable biomarkers present in halophiles include photosynthetic pigments, UV- and visible light absorbing compounds, compatible solutes and specific lipids. Raman spectroscopy is an excellent tool for detection of microbial pigments (Jehlička *et al.*, 2013). Developing methods of trace analysis of biomarkers in minerals using Raman microspectrometry is of crucial importance for understanding their conservation and transformation during the time. Terrestrial field detection of biomarkers using miniature Raman spectrometers in Mars-analogue sites on Earth can be seen as a training for next Martian astrobiological missions. In fact both the European Space Agency (Exomars) and the North American Space Agency (Mars 2020) robotic rovers will include Raman spectrometers. Sedimentary evaporitic series in the actual geological record are remnants of ancient hypersaline environments. Those systems are formed by minerals from the groups of chlorides, sulfates or borates. Residues of biomass of halophiles and biomarkers such as carotenoids can be detected and discriminated using Raman spectrometry. Examples of different approaches are given here: a) investigations of individual inclusions using Raman microscopic tools and b) simultaneous detection of minerals and biomarkers using portable Raman spectrometers. Biomarkers of halophiles in their natural environments as well as those of halophiles incorporated in synthetic crystals of salts are investigated.

Keywords: crystalline salts, halophiles, inclusions, Raman spectroscopy

Acknowledgements. This work was suported by Czech Science Foundation (17-04270S)

References

Jehlička, J., Edwards, H.G.M., & Oren A. (2014). Raman spectroscopy of microbial Pigments. *Appl Environ Microbiol* **80**: 3286–3295.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 73)

Biotechnological potential of halophilic archaea isolated from Romanian extreme habitats

Gabriela Teodosiu[1,✉] and Mădălin Enache[1]

[1]*Institute of Biology Bucharest of Romanian Academy, 296 Splaiul Independentei, 060031 Bucharest, P.O. Box 56-53, Romania;*
✉***Corresponding author, E-mail: gabriela.teodosiu@ibiol.ro.***

Abstract
In the last years, the extremely halophilic *Archaea* (haloarchaea) have become a focus of scientific interest owing to their unique properties in terms of physiology, ecology, biochemistry and molecular biology. Haloarchaea form dominant populations in hypersaline environments such as salt lakes, salterns, salt mines and salty soils, where the salt concentration exceeds 200-250 gl^{-1}, or reaches NaCl saturation. Apart from their evolutionary and ecological significance, haloarchaea have promising biotechnological applications. Hypersaline habitats are widely distributed in Romania and represented by salt lakes or underground salt deposits from Slănic Prahova, Ocna Sibiului, Sovata-Praid, Turda, Buzău, Brăila, Râmnicu Vâlcea, etc. The aim of our studies was to evaluate the biotechnological potential of some *Haloarcula* and *Halorubrum* strains regarding the synthesis of exopolysaccharides (EPS) and extracellular hydrolytic enzymes (amylase, protease, esterase, lipase, pectinase, inulinase, cellulase), halocins production and their ability to grow in media with crude oil. The methods used to test the activities of enzymes listed above were those described in early studies. The results revealed that the majority of isolates were able to synthesize different hydrolytic enzymes (pectinase, amylase, protease, esterase). The combined hydrolytic activity was also detected in some haloarchaea strains. Two strains produced halocins which were active against other haloarchaea isolated from the same lake. The tested strains produced EPS in media with various carbon sources (glucose, fructose, sucrose, mannose). The haloarchaea isolated from Bride Cave Lake and from salt crystals have the ability to grow in the presence of 2 and 4 % crude oil.

Keywords: biotechnology, haloarchaea, Romanian hypersaline habitats

Acknowledgements. This work was suported by project no. RO1567-IBB05/2018 from the Institute of Biology Bucharest of Romanian Academy.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 74)

Coproduction of alpha amylase and polyhydroxyalkanoate by *Halococcus* strain GUVSC8

Bhakti B. Salgaonkar[1],✉ and Judith M. Bragança[1],✉

[1]*Dept. of Biological Sciences, Birla Institute of Technology and Science Pilani, K K Birla, Goa Campus, NH-17B, Zuarinagar, Goa, India-403 726;* ✉***Corresponding authors, E-mail: Judith@goa.bits-pilani.ac.in; salgaonkarbhakti@gmail.com***

Abstract

Isolate GUVSC8 was obtained from commercial salt crystals of Vedaranyam, Tamil Nadu, India and categorized as an extreme halophile based on its requirement of 25% (w/v) NaCl for growth. Morphological, chemotaxonomic and 16S rRNA gene sequences revealed the cells to be coccus, producing bright orange C-50 bacterioruberin pigmentation with 95.95% similarity to *Halococcus hamelinensis* DQ017835. Culture was grown in minimal medium containing 1% (w/v) starch, the cell-free supernatant was used to extract amylase using cold acetone whereas, the cell pellet was used to extract polyhydroxyalkanoates. The polymer was extracted using sodium hypochlorite-chloroform and characterized to be a co-polymer of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) using crotonic acid assay, FT-IR, DSC, ^{1}H NMR analysis. Up on incubation of the crude enzyme with starch, produced numerous pores on the starch granules, indicating vigorous starch degradation and confirming the amylolytic activity. TLC and LC-ESI-MS analysis revealed, the major end product of starch hydrolysis to be glucose, maltose, maltotriose, maltotetrose, maltopentose and other maltooligosaccharides, thus confirming it to be an alpha-amylase. Effects of NaCl, pH and temperature, on activity of partially purified amylase, revealed best amylase activity at 2M NaCl, pH6 and 45°C. The amylase was stable and active in presence of divalent cations such as Mg^{2+}, Ni^{2+}, Zn^{2+}, Ca^{2+} and Co^{2+}. The activity increased in presence of Mn^{2+} and it lost 87% of its activity in presence of EDTA. To the best of our knowledge, this is the first report on coproduction of alpha amylase and PHAs by a salt crystal isolate belonging to the genus *Halococcus*.

Keywords: alpha-amylase, Archaea, *Halococcus*, halophiles, polyhydroxyalkanoates.

Acknowledgements. B.B.S acknowledge the financial help provided by Council of Scientific and Industrial Research (CSIR) India towards Research Assciateship (RA; Ref No: 09/919(0030)/2016-EMR-I).

Halo-thermophilic protease from *Halococcus agarilyticus* GUGFAWS-3 (MF425611)

Sanket K. Gaonkar[1] and Irene Furtado[1,✉]

[1]Goa University, Faculty of Life science, Department of Microbiology, Taleigao Plateau, Panjim-Goa, India; ✉***Corresponding author, E-mail: ijfurtado@unigo.ac.in***

Abstract

Halococcus agarilyticus GUGFAWS-3 is an archaeon isolated from a marine, white sponge (*Haliclona* sp.) inhabiting rocks in the intertidal region of Anjuna, Goa, India. Its cells have glycerol diether moieties and R-O-R and long isoprenoid chains, detectable in FTIR analysis, and have 99.1% 16S rRNA gene similarity to *Halococcus agarilyticus* 62E^T of the family *Halococcaceae* (Gaonkar & Furtado, 2018). Uniquely, this organism simultaneously produces halo-extremozymes in 25 % NaCl, corresponding to 49.5 U mL^{-1} of protease and 3.67 U mL^{-1} of lipase. The halophilic-protease is constitutively produced at low levels regardless of the availability of substrates (Kalisz, 1998). The enzyme was partially purified by ethanol precipitation, dialysis and Sephadex G-200 gel filtration chromatography to 12.26 fold and is active over a broad range of temperatures (30-80°C); NaCl concentrations (0- 5 M) and pH (3-13), with optimum activity at 70°C, 3 M NaCl and pH 7. The enzyme is thermo-stable at 70 °C for 120 h, retaining 40 % of its activity. Furthermore, it is stable in polar and non-polar organic solvents, detergents and hydrocarbons. Metal cations enhance its activity in the order of $Ca^{2+} > Ni^{2+} > Fe^{3+} > Co^{2+} > Mg^{2+} > Cu^{2+} > Mn^{2+}$. Partially inhibited by β-mercaptoethanol, PMSF and EDTA, the enzyme is a cysteine, serine and metal dependent protease. We report here a neutral halo-thermophilic protease. This is the first time such an extremozyme is reported from strain GUGFAWS-3 and also for the genus *Halococcus*. Efforts to study its various biotechnological potentials will also be highlighted and discussed.

Keywords: haloarcheon, halo-extremozyme, halostable, marine *Haliclona* sp., protease.

Acknowledgements. S.A. Gaonkar is gratetful for a Goa University Research Studentship.

References

Gaonkar, S. K., & Furtado, I. J. (2018). Isolation and culturing of protease-and lipase-producing *Halococcus agarilyticus* GUGFAWS-3 from marine *Haliclona* sp. inhabiting the rocky intertidal region of Anjuna in Goa, India. *Ann Microbiol* **68**(12): 851-861.

Kalisz, H. M. (1988). Microbial proteinases. In *Enzyme studies* (pp. 1-65). Springer, Berlin, Heidelberg.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 76)

Single amplified genomes from Red Sea brine pool extremophiles - a source of novel enzymes

Ram Karan[1,✉], Stefan Grötzinger[1], Malvina Vogler[1], Anastassja Akal[1], Dominik Renn[1], Alexandra Vancea[1], Shiladitya DasSarma[2], Jörg Eppinger[1] and Magnus Rueping[1,✉]

[1]*King Abdullah University of Science and Technology, Thuwal, Kingdom of Saudi Arabia;*
[2]*University of Maryland School of Medicine, Baltimore, MD, USA;* ✉***Corresponding authors, E-mail: ram.karan@kaust.edu.sa , magnus.rueping@kaust.edu.sa.***

Abstract

Enzymes originating from harsh environments offer exceptional stability under extreme conditions and are therefore highly in demand for industrial applications. However, understanding and harnessing of life under extreme conditions are challenging due to the difficulties of *in situ* observation and the lack of cultivatable organisms (> 99% can't be cultivated). We used culture-independent methods to comprehensively assess the structure and function of polyextremophilic enzymes from brine pools located at the bottom of the Red Sea. The identified extremozymes - alcohol dehydrogenases, carbonic anhydrases, proteases, glucose dehydrogenase, and 2-hydroxy dehydrogenase were bioinformatically selected from single amplified genomes (SAGs) for their biotechnological potential. The extremozymes were expressed in *Escherichia coli* or *Haloferax volcanii* and/or *Halobacterium* sp. NRC-1. Indeed, purified enzymes showed activity under extreme temperature, salt, and organic solvents. Our crystal structures of extremozymes combined with biochemical analyses provide insights into how enzymes adopt to these extreme conditions. The findings will facilitate the bioengineering of enzymes with valuable properties for biotechnology. The methodology provides a general guide for scientific and industrial investigations of the enzymatic treasure of the 'microbial dark matter'.

Keywords: extremophiles; extremozyme; halophiles; polyextremophiles; thermophiles.

Acknowledgements. This work was supported by King Abdullah University of Science and Technology (KAUST).

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 77)

Capacity of *Halomicrobium mukohataei* DSM 12886 to overcome silver-induced oxidative stress

Doriana-Mădălina Buda[1], Paul-Adrian Bulzu[1,2],
Lucian Barbu-Tudoran[1,3], Alina Porfire[4], Laura Pătraș[1,2],
Alina Sesărman[1,2], Septimiu Tripon[1,3] and Horia L. Banciu[1,✉]

*[1]Babeş-Bolyai University, 400006, Cluj-Napoca, Romania; [2]Institute for Interdisciplinary Research in Bio-Nano-Sciences, 400271, Cluj-Napoca, Romania; [3]National Institute for Research and Development of Isotopic and Molecular Technologies, 400293 Cluj-Napoca, Romania; [4]University of Medicine and Pharmacy „Iuliu Hatieganu", 400012, Cluj-Napoca, Romania; ✉**Corresponding author, E-mail: horia.banciu@ubbcluj.ro***

Abstract

Toxic levels of heavy metals pose a significant threat on living cells, triggering metabolic strategies that help in adaptation to heavy metal-induced stress. We explored the mechanisms assisting the silver resistance in the extremely halophilic archaeon *Halomicrobium mukohataei* DSM 12286. The modulatory effects of $AgNO_3$ on the cellular antioxidant systems were assessed by evaluating the malondialdehyde levels, the catalase activity, and the total antioxidant, non-enzymatic capacity. Biosynthesized silver nanoparticles were characterized using UV-VIS spectroscopy, bright-field microscopy, transmission and scanning electron microscopy, energy-dispersive X-ray spectroscopy, dynamic light scattering, and the zeta potential measurement. Additionally, bioinformatic approaches were used to infer putative Cu/Ag-transporters that might mediate the evidenced intracellular accumulation of silver nanoparticles. *H. mukohataei* was seemingly able to mitigate Ag-induced oxidative stress mainly by using non-enzymatic cellular antioxidants. Additionally, we noted the synthesis of spherical and electrically stable intracellular nanoparticles, with sizes ranging from 20 to 100 nm. It was further speculated that active Ag-resistance in *H. mukohataei* could be achieved through Ag^+ uptake by P_{IB} type ATPases followed by accumulation of silver in its colloidal form. To the best of our knowledge, the present work is the first documentation of intracellular silver accumulation in halophilic *Archaea*, thus broadening current knowledge on heavy-metal resistance in salt-loving microbes, with implications for their potential uses in biomining or bioremediation strategies.

Keywords: bioremediation, *Halomicrobium*, oxidative stress, silver resistance.

Acknowledgements. This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS–UEFIS-CDI, project number PN-III-P4-ID-PCE-2016-0303.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 78)

Heterologous low-salt production of ectoine and hydroxyectoine: is *Escherichia coli* the answer?

Lukas Bethlehem[1,✉], Katharina Domenika Moritz[1],
Moritz Lucius Sümmermann[1], Peter Schein[2] and Erwin Arno Galinski[1]

[1]*Institute of Microbiology & Biotechnology, Meckenheimer-Allee 168, 53111 Bonn, Germany;*
[2]*Institute of Biochemistry & Molecular Biology, Nussallee 11, 53111 Bonn, Germany;*
✉***Corresponding author, E-mail: l.bethlehem@uni-bonn.de.***

Abstract

The compatible solute ectoine is the most successful biotechnological product of halophilic *Bacteria*. It is at present still produced industrially with the halophilic *Halomonas elongata* (bitop-AG, Dortmund, Germany). Because of an ever increasing demand and the inherent draw-back of separating the product from a salt-containing fermentation broth, heterologous production has become an issue. However, numerous attempts using either *Escherichia coli* or *Corynebacterium glutamicum* as a chassis have remained unsatisfying. We have for the first time applied the ectoine/hydroxyectoine biosynthetic gene cluster from a non-halophilic donor, namely the acidophilic *Acidiphilium cryptum*, expecting prime performance of its enzymes even at low salinity. In comparison with other heterologous processes published so far, we achieved an unchallenged maximal specific production rate of 345 mg ectoine/(g dcw x h) in shaking-flasks with glycerol as carbon source. Biosynthesis of the product, which was excreted into the medium, was so efficient that its specific production amounted to 2.9 g/g biomass. Using ^{13}C-flux analysis with a novel inhouse software solution, we were able to identify PEP-carboxylase as the key reaction for anaplerotic carbon flux into the product. Further quantitative calculations revealed that we achieve 66% of the theoretical maximal yield. In order to comprehend potential limitations in the scale-up process, a systems approach will be presented including plasmid copy number, mRNA (pPCR) and protein abundance (mass spectrometry) determinations as well as metabolomic studies (GC-MS).

Keywords: *Acidiphilium cryptum*, compatible solute, *Escherichia coli*, heterologous production, hydroxyectoine/ectoine.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 79)

Got culture? Halophiles for teachers, students and our communities

Jaimi Butler[1,✉] and Bonnie K. Baxter[1]

[1]*Great Salt Lake Institute, Westminster Collegen, 1840 South 1300 East, Salt Lake City, Utah, USA;*
✉***Corresponding author, E-mail: jbutler@westminstercollege.edu.***

Abstract

It is common to hear school teachers complain regarding teaching about microorganisms. Their students often say, "I cannot see them!" This is a challenge in teaching about the invisible microbial world. And then you are fortunate enough to visit a place like Great Salt Lake (Utah, USA) where the vibrant pink and green colors of its bays captivate people from all over the world even as they fly into the nearby airport, viewing the nearby salt ponds and lake bays. These visitors may then be lucky enough to smell microbial byproducts known as "Lake stink." Great Salt Lake Institute (GSLI) at Westminster College strives to connect people to our important salty neighbor through research and education, and halophilic microorganisms, "halophiles," are the foundation of our programs. This presentation will underscore the value of halophiles in educational settings and dispel the myth that it is hard to understand them. At GSLI, halophiles have been a featured component of undergraduate research, K-12 curriculum development for classrooms, arts integration with science, and field-work. These valuable research models, when placed in the context of their environment, become charismatic microfauna, engaging students, researchers and the broader community. You could say we are giving our community culture!

Keywords: community engagement, halophiles, science education.

Acknowledgements. This work was suported by Westminster College, W.M. Keck Foundation and NASA.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 80)

The story of ectoine

Erwin A. Galinski[1,✉]

[1]*Institute of Microbiology & Biotechnology, Meckenheimer Allee 168, 53115 Bonn, Germany;*
✉***Corresponding author, E-mail: galinski@uni-bonn.de***

Abstract

The novel amino acid derivative ectoine was discovered in 1985 as a minor compatible solute in the bacterial genus *Ectothiorhodospira* (Galinski *et al*., 1985). It has since been detected in an ever-growing number of bacterial species (to date more than 400) and a few archaeal genera (Widderich *et al*., 2016), but also in some eukaryotic protists (Harding *et al*., 2016). Due to its apparently unique stabilizing and protecting properties, ectoine has found numerous applications as a medical product in the skin and health care sector and is at present produced industrially with the halophilic *Halomonas elongata* at several tons per year (Kunte *et al*., 2014). This presentation recalls the story of ectoine from an exotic find to a sought-after natural product and the people involved in its success. We will see what molecular features make this molecule special in its interaction with proteins, DNA and membranes and address potential medical applications. In this context I will also include the latest discoveries, which will provide new insights into ectoine´s novel molecular functions.

Keywords: compatible solute, ectoine, medical device, molecular interaction

References

Galinski, E. A., Pfeiffer, H.-P., & Trüper, H. G. (1985). 1,4,5,6-Tetrahydro-2-methyl-4-pyrimidinecarboxylic acid: a novel cyclic amino acid from halophilic phototrophic bacteria of the genus *Ectothiorhodospira*. *Eur J Biochem* **149**: 135-139.

Harding, T., Brown, M. W., Simpson, A. G. B., & Roger, A. J. (2016). Osmoadaptive strategy and its molecular signature in obligately halophilic heterotrophic protists. *Genome Biol Evol* **8**(7): 2241-2258.

Kunte, H. J., Lentzen, G., & Galinski, E. A. (2014). Industrial production of the cell protectant ectoine: protection mechanisms, processes, and products. *Curr Biotechnol* **3**(1): 10-25.

Widderich, N., Czech, L., Elling, F. J., Könneke, M., Stöveken, N., Pittelkow, M., Riclea, R., Dickschat, J. S., Heider, J., & Bremer, E. (2016). Strangers in the archaeal world: osmostress-responsive biosynthesis of ectoine and hydroxyectoine by the marine thaumarchaeon *Nitrosopumilus maritimus*. *Environ Microbiol* **18**(4): 1227-1248.

POSTER ABSTRACTS

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 83)

Biodiversity of the halophilic microbiome of the rhizosphere of *Salicornia*

Shahjahon A. Begmatov[1,2,✉], Olga V. Selitskaya[2],
Natalia A. Manucharova[3], Lina V. Vasilyeva[1], Julia J. Berestovskaja[1] and
Natalia V. Drenova[4]

[1]Winogradsky Institute of Microbiology, Russian Academy of Sciences, 60 let Oktjabrja pr-t, 7, 117312, Moscow, Russia; [2]Russian State Agrarian University - Moscow Timiryazev Agricultural Academy 49, Timiryazevskaya str., 127550, Moscow, Russia; [3]Moscow State University named after M.V. Lomonosov 1, Leninskie Gory str., 119991, Moscow, Russia; [4]All-Russian Centre for Plant Quarantine, 32, Pogranichnaya str., 140150, Moscow region, Russia;
✉Corresponding author, E-mail: shoxabdullayevich@gmail.com.

Abstract

The drying-out of the Aral Sea Basin is considered a catastrophic ecological problem of Central Asia and caused massive salinization and desertification of lands. Many halotolerant and halophilic bacteria were isolated and detected in hypersaline soils of arid regions, but their taxonomic description has not yet been completed, and their distribution and ecology have not yet been fully studied in detail (Oren, 2011). Our studies were focused on examining the biodiversity and ecology of the rhizosphere microbiome of *Salicornia*, a halophytic plant widely distributed in this region, and host to many representatives of halophilic microorganisms, to characterize the role of the halophilic microbiome in the growth of the host plant. To achieve this goal we carried out Illumina sequencing of the V4 hypervariable region of the 16S rRNA genes amplified from total DNA of selected soils. The experiments were performed according to standard methods. The results show that the major taxonomic groups of halophilic bacteria belong to the genera *Marinococcus* (2%), *Alcalibacterium* (1%), and *Halomonas* (3%), to the *Actinobacteria* (2%), and to the families *Alteromonadales* (2%), *Nitriliruptoraceae* (2%), and *Desulfobulbaceae* (1%). Representatives of the archaeal domain included several genera: *Natronococcus* (4%), *Haloterrigena* (3%), *Halovivax* (2%), *Halorubrum* (2%), and *Halomicrobium* (2%). The research of the diversity and ecology of halophilic bacteria and archaea in the *Salicornia* rhizosphere provides a theoretical concept and knowledge, while also offering future prospects to evaluate and develop potential biotechnological applications.

Keywords: Aral Sea, rhizosphere, *Salicornia*, salinity.

References

Oren, A. (2011). Ecology of halophiles. In: Horikoshi K. (ed). Extremophiles handbook, 343-361. Springer, Tokyo.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 84)

Characterization of halophyte rhizosphere microbiomes at Great Salt Lake, Utah

Emily G. Calhoun[1], Ambur M. Staab[1] and David L. Parrott, Jr.[1,✉]

[1]Westminster College, 1840 S 1300 East, Salt Lake City, UT 84105, USA;
✉Corresponding author, E-mail: dparrott@westminstercollege.edu.

Abstract

The saline soils comprising the shoreline of Great Salt Lake, Utah (GSL) provide a unique habitat for both halophytes (salt-tolerant plants) and the microorganisms that inhabit their rhizosphere. While plant diversity has been well documented at GSL, little is known about the microbial diversity in the rhizosphere. Here we present preliminary data characterizing the halophyte rhizosphere microbiome at two GSL locations; the more saline North Arm near the artwork, Robert Smithson's *Spiral Jetty* (SJ), and the less saline South Arm on Antelope Island. The rhizosphere of several plants along the shoreline at both SJ and Antelope Island locations was sampled. For each sample, plants were identified, soil salinity was quantified, DNA was isolated, and microbial cultures were established on either MGM or TSA media. Numerous unique isolates were observed on both media, indicating the presence of both halophiles and non-halophiles in the rhizosphere. Subsequent 16S rRNA gene sequencing substantiated this, identifying a combined total 58 species of Archaea and more than 1100 bacterial species among all collected samples. Our data suggest differences in the composition of rhizosphere microbiomes depending on location, soil type and salinity, and plant species. Decreased diversity of both archaeal and bacterial species was observed in rhizospheres at SJ compared to Antelope Island. Interestingly, a corresponding increase in the representation of halophilic Archaea at SJ was observed, possibly linked to the much higher salt concentration in the North Arm. Our results provide insight into the halophyte rhizosphere microbiome and expand our current knowledge of halophyte-halophile relationships.

Keywords: Great Salt Lake, halophile, halophyte, rhizosphere, soil microbiome

Acknowledgements. This work was suported by Westminster College and the Great Salt Lake Institute.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 85)

Halonotius species genome-scale metabolic reconstructions of new designated cobalamin synthesis as a Black Queen (BQ) function in haloarchaea

Ana Durán-Viseras[1,✉], Adrian-Ștefan Andrei[2], Rohit Ghai[2],
Cristina Sánchez-Porro[1] and Antonio Ventosa[1]

[1]University of Sevilla, Faculty of Pharmacy, Department of Microbiology and Parasitology, Sevilla, Spain; [2]Biology Centre of the Czech Academy of Sciences, Institute of Hydrobiology, Department of Aquatic Microbial Ecology, České Budějovice, Czech Republic;
✉Corresponding author, E-mail: anaduran@us.es.

Abstract

Hypersaline aquatic and terrestrial ecosystems display a cosmopolitan distribution. These environments teem with microbes and harbour a plethora of prokaryotic lineages that evaded ecological characterization due to the prior inability to cultivate them or to access their genomic information. In order to close the current knowledge gap, we performed two sampling and isolation campaigns in saline soils of the Odiel Saltmarshes and the salterns of Isla Cristina (Huelva, Spain). From the isolated haloarchaeal strains subjected to high-throughput phylogenetic screening, two were chosen (F9-27 and F15B) for physiological and genomic characterization because of their relatedness to the genus *Halonotius*. The topology of the phylogenomic tree based on 257 conserved protein sequences showed agreement with the phylogenetic ones based on 16S rRNA and *rpoB´* genes, and together with average amino acid and nucleotide identities designated the two strains as novel species within the genus. We propose the names *Halonotius roseus* sp. nov. and *Halonotius terrestris* sp. nov. for the newly described strains. Comparative genomic analyses within the genus highlighted a typical ”salt-in” signature (characterised by acidic proteomes with low isoelectric points) and indicated heterotrophic aerobic lifestyles. Genome-scale metabolic reconstructions revealed that the newly proposed species, together with environmentally-recovered metagenome-assembled representatives, encode all the necessary enzymatic reactions involved in cobalamin (vitamin B_{12}) biosynthesis. Based on the worldwide distribution of the genus and its high abundance in hypersaline habitats we postulate that its members perform a critical Black Queen function within the halophilic communities.

Keywords: Black Queen function, cobalamin, *Halonotius*, metabolic reconstruction, phylogenomics.

Acknowledgements. This work was suported by a predoctoral fellowship (FPU) from the Spanish Ministry of Education, Culture and Sports and from a FEMS research and training grant and founded by projects CGL2017-83385-P (MINECO) and BIO-213 (Junta de Andalucía), both with FEDER funds.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 86)

Description of the eukaryotic microbial population in a solar saltern pond of the Odiel marshlands (SW Spain)

Patricia Gómez-Villegas[1,✉], Antonio León-Vaz[1], Marta Vila[1],
Javier Vigara[1] and Rosa León[1]

[1]Laboratory of Biochemistry and Molecular Biology, Faculty of Experimental Sciences, Marine International Campus of Excellence (CEIMAR), University of Huelva, 21071 Huelva, Spain;
✉Corresponding author, E-mail: patgomvil@gmail.com.

Abstract

Halophiles are widespread in the three domains of life; nevertheless, there are few halophilic eukaryotes able to thrive in extreme salinity conditions in comparison to prokaryotes. For that reason, most research on microorganisms adapted to hypersaline habitats has been focused on Archaea and Bacteria, with microbial eukaryotes receiving much less attention. Hereby, we expose a research about eukaryotic microorganisms living in a solar saltern pond located in the Odiel marshlands (Huelva), at the southwest of Spain. In this work, we used a culture-independent method to study the eukaryotic population in the brine ponds at 33% salinity. We constructed and characterized a library of PCR amplified 18S rRNA genes with genomic DNA extracted from a water sample as template. In addition, we isolated several microalgae strains and we identified them by PCR amplified 18S rRNA gene sequence comparison with DNA databases. Finally, we studied their pigments composition by High Performance Liquid Chromatography. This study means the first characterization of the eukaryotic microbial diversity of the extreme saline water of this environment. We found diverse microalgae belonging to the *Dunaliella* genus, and various protozoan predators related to the *Colpodella* genus. Concerning the pigments, we found that the isolated microalgae produced neoxanthin, violaxanthin, antheraxanthin, lutein, chlorophyll *a*, chlorophyll *b*, and β-carotene. Following studies will be focused on the bioactive capacity of these pigments, testing their antitumor, antimicrobial and antioxidant activities.

Keywords: carotenoids, microalgae, protist, saltern, 18S rRNA clone library.

Acknowledgements. This work was suported by INTERREG VA España–Portugal (POCTEP) 2014–2020 Cooperation Program, grant number 0055_ALGARED_PLUS_5_E and SUBV. COOP. EURO-REGION ALENTEJO-ALGARVE-ANDALUCIA 2018- JA.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 87)

Characterization of the prokaryotic diversity inhabiting a solar saltern pond of the Odiel marshlands (SW Spain)

Patricia Gómez-Villegas[1,✉], Javier Vigara[1] and Rosa León[1]

[1]*Laboratory of Biochemistry and Molecular Biology, Faculty of Experimental Sciences, Marine International Campus of Excellence (CEIMAR), University of Huelva, 21071 Huelva, Spain;*
✉***Corresponding author, E-mail: patgomvil@gmail.com.***

Abstract

Halophilic microorganisms can be an excellent source of useful compounds for industrial applications. However, the microbial population inhabiting extreme saline environments has been under-estimated, due to difficulties encountered in the isolation and cultivation of these microorganisms. The solar salterns located in the Odiel marshlands (Huelva), at the southwest of Spain, are an excellent example of a poorly explored hypersaline environment. In this work, we used two culture-independent methods to study the prokaryotic diversity present in the brine ponds at 33% salinity. We constructed and characterized libraries of PCR-amplified 16S rRNA genes with genomic DNA extracted from a water sample as template; and we used 16S rRNA Next-Generation Sequencing (NGS) based on the Illumina MiSeq platform to profile the same genomic sample. Furthermore, the ability of this microbial population to excrete haloenzymes was tested on agar plates. This work entails the first study of the aquatic microbial composition in this environment, where we found only one bacterial species, *Salinibacter ruber*, and a high diversity of archaea. Moreover, we demonstrated that NGS and clone library approaches were comparable regarding the estimation of the major genera found in the sample, including *Halorubrum*, *Haloquadratum*, *Halonotius* and *Halobellus.* Finally, we showed that the biomass presented different hydrolase activities, comprising α-amylase, protease, lipase/esterase, cellulase and laccase. Further studies will focus on the isolation and identification of the strains with the higher activity for each enzyme, followed by the optimization of the parameters that enable the best activity.

Keywords: archaea, haloenzymes, Odiel marshlands, 16S rRNA metagenomics.

Acknowledgements. This work was suported by INTERREG VA España–Portugal (POCTEP) 2014–2020 Cooperation Program, grant number 0055_ALGARED_PLUS_5_E and SUBV. COOP. EURO-REGION ALENTEJO-ALGARVE-ANDALUCIA 2018- JA.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 88)

Bacillus alkalisalsus sp. nov., isolated from saline-alkaline soil samples

Guohong Liu[1], Jieping Wang[1], Xuefang Zheng[1] and Bo Liu[1,✉]

[1]*Agricultural Bio-resources Research Institute, Fujian Academy of Agricultural Sciences, Fuzhou city, 350003, Fujian Province, China;* ✉***Corresponding author, E-mail: fzliubo@163.com***

Abstract

From a survey of the diversity of *Bacillus*-like species, a novel strain FJAT-45037 was found to be differing from the known *Bacillus* species. In this study, the taxonomic status of this potential new species was determined using genomics-based polyphasic taxonomy (Ramasamy *et al.*, 2014). Cells of strain FJAT-45037 were aerobic, endospore-forming and motile. Temperature range for growth was 10 to 35 °C (optimum 30 °C). pH range for growth was 7.5~12.0 (optimum 9.0~10.0). NaCl concentration range for growth was 0-12% (w/v) (optimum 1%). The 16S rRNA gene sequence-based phylogenetic analysis revealed that strain FJAT-45037 is most closely related to *Bacillus marmarensis* GMBE 72^T (97.67%), and their DNA-DNA hybridization (*is*DDH) value was 18.6% with an average nucleotide identity (ANI) value of 84.46%. The DNA G+C content was 39.79 mol%. The major cellular fatty acids were anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. The diagnostic diamino acid of the cell-wall peptidoglycan was *meso*-diaminopimelic acid. MK-6 and MK-7 were the predominant quinones. Major polar lipids were diphosphatidyl glycerol, phosphatidyl glycerol and phosphatidyl ethanolamine. On the basis of the polyphasic evidence presented, the strain was considered to represent a novel member of the genus *Bacillus*, for which the name *Bacillus alkalisalsus* sp. nov. was proposed. The type strain is FJAT-45037 (= DSM 104053 = CCTCC AB 2016229).

Keywords: alkalitolerant, *Bacillus alkalisalsus*, genome, polyphasic taxonomy.

Acknowledgements. This work was suported by the Science and Technology Innovation Team Program of Fujian Academy of Agricultural Sciences (STIT 2017-1-11)

References

Ramasamy, D., Mishra, A. K., Lagier, J. C., Padhmanabhan, R., Rossi, M., et al. (2014). A polyphasic strategy incorporating genomic data for the taxonomic description of novel bacterial species. *Int J Syst Evol Microbiol* **64**:384-391.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 89)

Bacillus alkalisoli sp. nov., isolated from alkaline soils

Guohong Liu[1], Xuefang Zheng[1], Jieping Wang[1], Jianmei Che[1] and
Bo Liu[1,✉]

[1]*Agricultural Bio-resources Research Institute, Fujian Academy of Agricultural Sciences, Fuzhou city, 350003, Fujian Province, China;* ✉***Corresponding author, E-mail: fzliubo@163.com***

Abstract

The genus *Bacillus* belongs to the family *Bacillaceae* in the phylum *Firmicutes* and represents a large group of rod-shaped, aerobic or facultatively anaerobic, Gram- positive endospore-forming bacteria with low DNA G+C content (32–66 mol%) (Logan & De Vos, 2009). During a survey of the diversity of *Bacillus*-like species in western region of China, a novel strain FJAT-45086 was found that differs from the known *Bacillus* species. The genomics-based polyphasic taxonomy was used to identified the strain (Ramsamy *et al.*, 2014). Cells of strain FJAT-45086 were aerobic, endospore-forming and motile with a single polar flagellum. The growth requirements were: 15-35°C (optimum 30°C), pH 6-12 (optimum 9), with NaCl concentration 0-10% (optimum 4 %). The major cellular fatty acids were anteiso-$C_{15:0}$ and $C_{16:0}$. The phylogeny of 16S rRNA gene revealed strain FJAT-45086 had the highest similarity with *Bacillus hemicellulosilyticus* JCM 9152^T (96.7 %); their isDDH and ANI value was 22.7 % and 84.79 %, respectively. The DNA G+C content was 37.81 mol%. The diagnostic diamino acid of the cell-wall peptidoglycan was *meso*-DAP. MK-7 was the predominant quinone. Major polar lipids were DPG, PE, PG and PC. Based on polyphasic evidence presented, strain FJAT-45086 (= DSM 104056 = CCTCC AB 2016232) is considered as the type strain, for which the name *Bacillus alkalisoli* sp. nov. is proposed.

Keywords: alkalitolerant, *Bacillus alkalisoli*, genome; polyphasic taxonomy.

Acknowledgements. This work was suported by the Science and Technology Innovation Team Program of Fujian Academy of Agricultural Sciences (STIT 2017-1-11), the Fujian Special Fund for Public Interest Research (2018R1017-1).

References

Logan, N. A., & De Vos, P. (2009). Genus I. *Bacillus*. In Bergey's Manual of Systematic Bacteriology, vol. 3, pp. 21–128. Edited by De Vos P, Garrity G, Jones D, Krieg NR Ludwig W, Rainey FA, Schleifer KH, Springer, Whitman WB. New York:1-1450.

Ramasamy, D., Mishra, A. K., Lagier, J.C., Padhmanabhan, R., Rossi, M., *et al.* (2014). A polyphasic strategy incorporating genomic data for the taxonomic description of novel bacterial species. *Int J Syst Evol Microbiol* **64**:384-391.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 90)

Biodiversity and antibiotic resistance profiling of a Triassic halite deposit in Northern Ireland

Julianne Megaw[1,✉], Stephen Kelly[1], Thomas Thompson[1],
Timofey Skvortsov[1] and Brendan Gilmore[1]

[1]*School of Pharmacy, Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, UK;* ✉***Corresponding author, E-mail: j.megaw@qub.ac.uk.***

Abstract
Kilroot salt mine, a Triassic halite deposit, is the only hypersaline environment in Ireland. We profiled the culturable and nonculturable microbiota of this unstudied environment using conventional isolation (with the addition of some augmented techniques) and metagenomics. Based on 16S sequencing, 89 extremely halophilic archaea from six genera, and 55 halophilic and halotolerant bacteria from 19 genera were isolated. The archaea were similar to what has been previously isolated from other ancient halite deposits, and as expected, numerous additional genera were identified in the metagenome, indicating the limitations of culture-based approaches. We also observed high levels of phenotypic antimicrobial resistance (AMR) among the isolates. Annotation of whole genome and metagenomic sequence data identified a number of genes which might explain the observed resistant phenotypes, however there were fewer than expected, suggesting that these microorganisms may use novel, as yet unidentified resistance mechanisms. A growing body of evidence suggests AMR is not a modern phenomenon, but that its origins are ancient, long predating modern antibiotic use. Studying AMR in environments that have had little or no exposure to anthropogenic antibiotic sources provides a critical measure of its natural diversity, which has significant implications in our understanding of its prevalence and evolution.

Keywords: antibiotic resistance, haloarchaea, isolation, metagenome, microbiome.

Acknowledgements. This work was suported by the Irish Marine Institute under the Beaufort Marine Biodiscovery Research award funded by the Irish Government under the National Development Plan (2007–2013), the Biotechnology and Biological Sciences Research Council (BBSRC) through an Industrial CASE training grant (BB/L017083/1) with the Almac Group, and the Department for the economy (DfE), Northern Ireland, UK.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 91)

Halorientalis pallida sp. nov., a new extremely halophilic archaeon isolated from a marine saltern in Spain

Cristina Sánchez-Porro[1,✉], Ana Durán-Viseras[1] and Antonio Ventosa[1]

[1]*University of Sevilla, Faculty of Pharmacy, Department of Microbiology and Parasitology, Sevilla, Spain;* ✉***Corresponding author, E-mail: sanpor@us.es***

Abstract

Solar salterns are extreme environments in which salinity plays a very important role and influences their biodiversity. Our research group has been dedicated to the study of the inhabitants of these environments. Metagenomic studies have shown that many microorganisms that inhabit these environments have not been isolated in pure culture to date. Isolation of these new groups is the main objectives of our project. We have a wide collection of isolates from solar salterns of Isla Cristina (Huelva, Spain). This work focuses on the taxonomic description of one of these isolates, strain F13-25. Strain F13-25 forms colonies with pink pigmentation. It presents an optimal growth at a concentration of 25% (w/v) NaCl, pH 7.5 and 37°C. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain F13-25 is a member of the genus *Halorientalis*. We have sequenced the genome of strain F13-25 and we have analyzed it by comparing it with the genomes of the type strains of the species of the genus. The results of the GGDC and ortoANI, ANIb and ANIm of strain F13-25 and its related species showed percentages below the accepted threshold value for the delineation of prokaryotic species. It contains phosphatidylglycerol, phosphatidylglyceryl phosphate methyl ester and a glycolipid chromatographically identical to diethyl diglycosyl diether. The G + C content of the DNA is 65.7 mol% (genome). The phylogenetic, genomic, chemotaxonomic and phenotypic analyses show that strain F13-25 represents a new species of the genus *Halorientalis*, for which we propose the name *Halorientalis pallida* sp. nov.

Keywords: Archaea, genome, saltern, taxonomy.

Acknowledgements. This work was supported by a predoctoral fellowship (FPU) from the Spanish Ministry of Education, Culture and Sports and from a FEMS research and training grant, and funded by projects CGL2017-83385-P (MINECO) and BIO-213 (Junta de Andalucía), both with FEDER funds.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 92)

The prokaryotic diversity in cultures of halophilic phototrophic and heterotrophic protists

Elena Selivanova[1,✉], Yuri Khlopko[1] and Andrey Plotnikov[1]

[1]*Institute for Cellular and Intracellular Symbiosis of the Ural Branch of the Russian Academy of Sciences, 460000, Pionerskaya st, 11, Orenburg, Russia;* ✉***Corresponding author, E-mail: selivanova-81@mail.ru***

Abstract

Protists constitute an important component of microbial communities in the aquatic ecosystems. In microbial food webs phototrophic protists produce organic matter utilized by heterotrophic bacteria as food. Some phototrophic protists secrete substances suppressing bacterial growth or modulating bacterial quorum sensing. Heterotrophic protists consume prokaryotes changing their abundance and diversity. The effects of interactions between protists and prokaryotes are poorly understood in the hypersaline environments. The aim of the study was to characterize the prokaryotic communities associated with cultures of heterotrophic and phototrophic protists, using high-throughput sequencing. We used nine cultures of protists isolated from inland saline lakes (Orenburg region, Russia) and stored for years under laboratory conditions at 150 or 200‰ salinities. Phototrophic protists were represented by genera of green algae *Dunaliella* and *Asteromonas*, whereas heterotrophic protists belonged to the Heterolobosea group. Sequencing of 16S rRNA gene amplicons using MiSeq (Illumina) revealed that the prokaryotic communities associated with the heterotrophic protists were more diverse than those in association with the phototrophic protists. Only two classes, Gammaproteobacteria and Halobacteria, represented by a few genera, were found in all the cultures of phototrophic protists. Here, *Halorubrum* spp. and *Halovibrio* spp. were the most abundant genera. Gammaproteobacteria, Halobacteria, Sphingobacteriia, Deltaproteobacteria, Alphaproteobacteria classes, and Nanohaloarchaeota phylum were associated with halophilic heterotrophic protists. The obtained results suggest that taxonomic composition of associated halophilic prokaryotic communities differs drastically in cultures of phototrophic and heterotrophic protists.

Keywords: diversity, halophilic microbial community, high-throughput sequencing, protists.

Acknowledgements. This work was suported by RFBR (17-04-02079, 17-04-00135).

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 93)

The effect of salting on the bacterial load of holy water from Catholic churches

Martina Turk[1,✉], Vesna Podgrajšek[1] and Nina Gunde-Cimerman[1]

[1]*Department of Biology, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia;* ✉***Corresponding author, E-mail: martina.turk@bf.uni-lj.si***

Abstract

Holy water plays an important role in various religions. It is also recognized as a source of potentially pathogenic microorganisms. The holy water was linked to human infections caused by *Pseudomonas aeruginosa*, *Acinetobacter baumanii*, *Escherichia coli*, and other enterobacteria. In the Roman Catholic Church salt can also be blessed for use as a sacramental and is sometimes added to holy water. Holy water is kept in fonts usually located at the entrance to the church to enable people to bless themselves with it on entering, potentially introducing their skin microbiota into the water in the process of doing so. In order to better understand the influence of salt addition on the bacterial community of holy water and the potential risk posed by this water as a source of infection with pathogenic microorganisms, we studied the composition of cultivable aerobic bacterial community in holy water from fonts and reservoirs of selected Catholic churches in urban areas. Large part of isolates were typical human skin commensals from the genus *Staphylococcus* followed by *Pseudomonas* isolates. Selected isolates were also tested for resistance to antibiotics. The addition of salt is not sufficient as a prevention against microbial contamination of holy water. Appropriate hygiene measures like regular water exchange and a thorough cleaning of the fonts should be used regardless of the addition of salt.

Keywords: antibiotic resistance, bacteria, holy water, NaCl, pathogens

Acknowledgements. This work was suported by the Slovenian Research Agency (programme P1-0170).

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 94)

Mg^{2+}-rich bitterns host highly adapted fungi

Polona Zalar[1,✉], Ivana Strmecki[1], Janja Zajc[2], Cene Gostinčar[1] and Nina Gunde-Cimerman[1]

[1]University of Ljubljana, Biotechnical Faculty, Department of Biology, Večna pot 111, Ljubljana, Slovenia; [2]National Institute of Biology, Večna pot 111, Ljubljana, Slovenia;
✉Corresponding author, E-mail: polona.zalar@bf.uni-lj.si

Abstract

Bittern brines, the residual water after the precipitation of NaCl in solar salterns, are highly enriched with magnesium salts, mostly with $MgCl_2$. These brines were long considered sterile, as high concentrations of Mg^{2+} are toxic for the majority of biological systems. However, it was shown that bittern brines of the Sečovlje salterns (Slovenia) are not completely free of living microorganisms (Zajc et al., 2014). To isolate fungi from bitterns, we used three different isolation techniques: filtration, enrichment in liquid shaken cultures, and dilution to extinction in liquid standing cultures. Using all three techniques, we isolated 120 fungal strains and identified them using morphological and molecular markers. Their abundance and biodiversity was found to be much lower in comparison to NaCl-rich brines: out of 68 fungal genera recorded in Sečovlje brines, only representatives of ten were found also in bitterns: *Aspergillus*, *Alternaria*, *Cladosporium*, *Debaryomyces*, *Hortaea*, *Meyerozyma*, *Penicillium*, *Phaeotheca*, *Vishniacozyma*, and *Wallemia*. Beside these, we recorded for NaCl-rich brines yet unknown species: *Bullera alba*, *Cadophora luteo-olivacea*, *Cladosporium langeronii*, *Peroneutypa scoparia*, *Pseudotaeniolina globosa*, *Toxicocladosporium irritans*, and *Verrucocladosporium dirinae*. Selected isolates were tested for growth in media with $MgCl_2$, and some of them could grow at a concentration of 1.5 M $MgCl_2$. Mg-rich brines can be considered as a distinct extreme habitat harbouring a mycobiome that only partially overlaps with that of thalassohaline brine.

Keywords: bittern, brine, extremophiles, fungi, hypersaline.

References:

Zajc, J., Džeroski, S., Kocev D., Oren, A., Sonjak, S., Tkavc, R., & Gunde-Cimerman, N. (2014). Chaophilic or chaotolerant fungi: a new category of extremophiles? *Front Microbiol* **5**: 1–15.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 95)

Characterization of extracellular DNA, the forgotten fraction of hypersaline environments

Borja Aldeguer[1], María Dolores Ramos-Barbero[1], Cristina López[1], Fernando Santos[1], Ana Belén Martín-Cuadrado[1] and Josefa Antón[1,✉]

[1]*Alicante University, Science Faculty, Department of Physiology, Genetics and Microbiology, Alicante University, Carretera San Vicente del Raspeig, 03080. Alicante. España;*
✉***Corresponding author, E-mail: anton@ua.es***

Abstract

Extracellular DNA (eDNA) is the fraction of environmental DNA outside the cells which contains viral and dissolved DNA. eDNA can play an important function in horizontal gene transfer and as source of nutrients, two of the most relevant ecological processes that influence environmental evolution and dynamic population. While some studies in hypersaline sediment showed that eDNA reached a high concentration, studies in hypersaline water have not yet been performed. Thus, this is the first work that provides information about characteristics and possible functions of eDNA in hypersaline water. In our study we determined the concentration, size and origin of eDNA, focusing our efforts mainly on dissolved DNA that is the most unknown fraction of the eDNA. For this purpose, hypersaline water samples were collected from the crystalizer CR30, in the solar salterns of Santa Pola (Spain), one of the hypersaline environments studied in more detail. We purified and analyzed eDNA and we sequenced it using Illumina with the aim of determining its origin, GC content and metabolic genes. We were interested in determining the environmental role of dissolved DNA, so we hypothesized that it could act as a UV protector and we performed an experiment in which the microbial community of the cristalizer was exposed to UV radiation in the presence of different DNA concentrations.

Keywords: dissolved DNA, extracellular DNA, hypersaline water, viral DNA.

Acknowledgements. This work was supported by the Spanish Ministry project CGL2015-66686-C3-3-P and Alicante University fellowship for reasearch initiation.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 96)

Patterns of prokaryotic diversity in sediments of brackish to hypersaline salt lakes in Romania

Andreea Baricz[1,2], Cecilia Chiriac[2], Paul-Adrian Bulzu[1], Adorján Cristea[1], Zsolt G. Keresztes[1], Tudor Tămaș[1], Mircea Alexe[1] and Horia L. Banciu[1,]✉

[1]Babeş-Bolyai University, 400006, Cluj-Napoca; Romania; [2]NIRDBS Biological Research Institute, Republicii 48, 400015, Cluj-Napoca, Romania;
✉Corresponding author, E-mail: horia.banciu@ubbcluj.ro.

Abstract

In this work, we explored the structure of prokaryotic communities inhabiting the sediments of nine lakes with different salinities (from 84 to 344 g L^{-1}) located in Central and South-Eastern Romania. The 16S amplicon sequencing approach combined with geochemical measurements were employed to i) assess local molecular diversity of prokaryotic communities, ii) evaluate the geographical and environmental factors affecting the community composition among sediments of distinct geochemical conditions, and iii) infer key microbial players in the biogeochemical cycling of C, S, and Fe. High relative abundances of 16S rRNA gene reads were assigned to *Actinobacteria*, *Alphaproteobacteria*, *Bacteroidia*, *Clostridia*, *Deltaproteobacteria*, *Gammaproteobacteria*, *Mollicutes*, and *Rhodothermia* bacterial classes and to *Halobacteria*, *Thermoplasmata*, and *Woesearchaeia* among the *Archaea*. Highest richness and diversity were found in sediments containing 96 g L^{-1} and 300 g L^{-1} NaCl. Additionally, the analyzed saline sediments appeared to be inhabited by uncultured *Bacteria* (*Gracilibacteria*, *Marinimicrobia*, *Omnitrophicaeota*, *Patescibacteria*) and *Archaea* (*Altiarchaeota*, *Asgardarchaeota*, *Diapherotrites*, *Hadesarchaeota*) with possible crucial roles in C and S cycling. A microbially-driven Fe cycle could be speculated from the presence of *Zetaproteobacteria*-affiliated reads in five sediments with different salinities. In conclusion, we provided clues about the microbial diversity and its putative functional capabilities in poorly investigated, saline aquatic habitats.

Keywords: diversity, hypersaline, microbial dark matter, sediments.

Acknowledgements. We acknolwedge the grant of the Romanian National Authority for Scientific Research, CNCS–UEFISCDI, project number PN-III-P4-ID-PCE-2016-0303. In addition to the above project, Andreea Baricz was also supported by PDI-PFE-Ctr. No. 22/2018 and PN2019-2022/19270201 –Ctr. 25N BIODIVERS3-BIOSERV projects.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 97)

Extremophiles everywhere and the limits of microbial life

Emese Bartha[1], Terry J. McGenity[1], Boyd A. McKew[1,] and
Etienne Low-Decarie[2,]✉

[1]University of Essex, School of Biological Sciences, Wivenhoe Park, Colchester, CO4 3SQ, UK;
[2]Canadian Space Agency, 6767 Route de l'Aéroport, Saint-Hubert, QC J3Y 8Y9;
✉***Corresponding author, E-mail: etienne.decarie@gmail.com***

Abstract

Microorganisms capable of growing in extreme conditions were expected to be only found living in extreme environments. However, recent studies found extreme-tolerant microbes (extremotolerants) in non-extreme environments. What is the environmental limit of extremotolerants? Will they have a lower growth rate than endemic microbes in non-extreme environments? Here we sampled microbial communities from three rivers and their connected marine sites and enriched for extremophiles/extremotolerants in seven different media: yeast extract plus various high concentrations of $ZnCl_2$, $CuSO_4$ (metalotolerants), NaCl (halophiles/ halotolerants), $MgCl_2$ (chaophiles/ chaotolerants), sorbitol (osmophiles/ osmotolerants), HCl, and NaOH (acidophiles/ acidotolerants and alkaliphiles/ alkalitolerants, respectively). Samples were enriched in these seven stressor media separately, along with control samples enriched in yeast extract media. These communities enriched in stressor and control media were inoculated in a gradient of the same type of stressor. We found that stressor-enriched cultures had a higher tolerance to stress conditions and could grow at a wide range of stressor concentrations compared to control-enriched cultures that only grew at low stressor levels. These differences were especially apparent for NaCl and $MgCl_2$. Therefore, these stressor-enriched communities could potentially consist of previously dormant obligate extremophiles and/or facultative extremotolerants surviving/growing in non-extreme conditions. These findings further support the theory that microorganisms can be found far outside of their physiologically optimal habitat, and question whether extremotolerants living in non-extreme environments differ from their counterparts in extreme environments.

Keywords: chaophile, extremophile, extremotolerant, halophile, microbial biogeography.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 98)

Exploration of the prokaryotic diversity in the hypersaline and lithium-rich Salar de Uyuni, Bolivia by DNA metabarcoding

Götz Haferburg[1,✉], Janosch Gröning[2], Nadja Schmidt[3],
Juan Carlos Erquicia[4] and Michael Schlömann[1]

[1]Environmental Microbiology, TU Bergakademie, Leipziger Strasse 29, 09599 Freiberg, Germany, [2]Institute of Microbiology, Allmandring 31, D-70569 Stuttgart, Germany, [3]Institute of Hydrogeology and Environmental Geology, TU Bergakademie, Gustav Zeuner Straße 12, 09599 Freiberg, Germany, [4]Tomás Frías Autonomous University, Potosí, Bolivia;
✉***Corresponding author, E-mail: goetz.haferburg@ioez.tu-freiberg.de***

Abstract

The Salar de Uyuni, situated in the Southwest of the Bolivian altiplano, is the largest salt flat on earth. Brines of the athalassohaline hypersaline environment are rich in lithium and boron. Due to the ever-increasing commodity demand the exploitation of brines for metal recovery from the world's largest lithium reservoir is likely to increase substantially in the near future. Only few studies have investigated the composition of halophilic microbial communities in brine waters of the Salar. Here we report on the microbial diversity of brine pools in the East, locally denoted "Ojos del Salar". The brine is characterized by a salinity of 28%, slightly acidic pH and high lithium concentrations. Four pool communities were analysed by sequencing the V3–V4 region employing Illumina MiSeq technology. Subsequently, Mothur was used for sequence processing and data analysis. Metagenomic analysis revealed the occurrence of an exclusively archaeal community comprising 24 halobacterial genera including only recently identified genera like *Halapricum*, *Halorubellus* and *Salinarchaeum*. Despite the high diversity of the encountered halobacteria community, almost half of the *Halobacteria* assigned sequences (46%) could not be classified on the genus level under stringent filtering conditions. Thus, it seems likely to discover new, hitherto undescribed genera in this particular habitat of Salar de Uyuni.

Keywords: 16S rRNA metagenomics, Bolivian altiplano, halophilic extremophiles, hypersaline lake, Salar de Uyuni.

Acknowledgements. This work was suported by the KSB-Stiftung, Frankenthal, within the project "Metal recovery from brine waters".

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 99)

Culturable prokaryotic diversity from Sambhar hypersaline lake

Swapnil Kajale[1,2], Yogesh Shouche[2], Neelima Deshpande[1] and Avinash Sharma[2,✉]

*[1]Department of Microbiology, MES Abasaheb Garware College, Pune, Maharashtra, 411004, India; [2]National Centre for Microbial Resource-National Centre for Cell Science, Pune, Maharashtra, 411021, India; ✉**Corresponding author, E-mail: avinash@nccs.res.in, avinash.nccs@gmail.com.***

Abstract

Sambhar Salt Lake is a large inland hypersaline lake in India situated at the eastern fringe of Thar desert, Rajasthan. This wetland is designated as Ramsar site due to its biotic importance and also known as the major salt producing site in India. High salinity, alkalinity and UV radiation makes this athalassohaline lake an unique natural ecosystem to explore the extremophilic microbial diversity. The present work investigated the culturable fraction of haloalkaliphiles from this less explored habitat using multiple methods. Sediment samples were collected from five different sites based on their locations and ionic strengths. Prokaryotic diversity was studied using various growth media and later identified using 16S rRNA gene sequencing. Whole genome squencing of potential isolates was carried out. A total of 447 isolates were identified belonging to 3 archaeal and 5 bacterial families. We also identified isolates belonging to 8 archaeal and 3 bacterial genera as potential novel taxa. Altogether the cultivation strategies used in this study contributed in capturing major haloarchaeal and halobacterial diversity with potential novel taxa which will enrich the microbial resource centres.

Keywords: athalassohaline, haloalkaliphile, Sambhar salt lake, 16S rRNA gene.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 100)

The community of culturable halotolerant and halophilic *Bacillus*-like species in Fujian Province

Guohong Liu[1], Xuefang Zheng[1], Jieping Wang[1], Jianmei Che[1], Min Lei[1] and Bo Liu[1,✉]

[1]Agricultural Bio-resources Research Institute, Fujian Academy of Agricultural Sciences, Fuzhou city, 350003, Fujian Province, China; ✉***Corresponding author, E-mail: fzliubo@163.com***

Abstract

The genus *Bacillus*, belongs to phylum *Firmicutes*, family *Bacillaceae* and represents a large group of rod-shaped, aerobic or facultatively anaerobic, Gram-stain-positive and endospore-forming bacteria with low DNA G+C content (32–66 mol%) (Logan & De Vos, 2009). Fujian Province has the longest coastal length in China. The aim of this study was to understand the diversity of halotolerant and halophilic *Bacillus*-like species in salinity tolerance environment of Fujian Province. The *Bacillus*-like bacteria were isolated from soils from saline enviroment in Fujian Province by culturomics and preliminarily identified based on the 16S rRNA gene sequences. Seventy-six isolates were obtained from 43 soil samples, and identified as 12 species belonging to 4 genera. The plate counts range of *Bacillus*-like species was $0.50\text{-}1.26\times10^{3}$ cfu·g^{-1}. Based on their occurrence frequency, the *Bacillus*-like species were discriminated as the high frequency species middle frequency species and low frequency species (other six species). Through the NaCl tolerance experiment, 64.4% strains were grouped into the halotolerant bacteria, and 35.6% strains were moderate halophiles. Therefore, there were many *Bacillus*-like species with high halotolerant ability, which provide rich resources and theoretical basis for exploring and utilization of functional strains.

Keywords： *Bacillus*-like species, halotolerant and halophilic, community.

Acknowledgements. This work was suported by the Science and Technology Innovation Team Program of Fujian Academy of Agricultural Sciences (STIT 2017-1-11), the Fujian Special Fund for Public Interest Research (2018R1017-1), National Key R & D Program of China (2017YFD0201100).

References

Logan, N.A., & De Vos, P. (2009). Genus I. *Bacillus*. In Bergey's Manual of Systematic Bacteriology, vol. 3, pp. 21–128. Edited by De Vos P, Garrity G, Jones D, Krieg NR Ludwig W, Rainey FA, Schleifer KH, Springer, Whitman WB. New York:1-1450.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 101)

Archaeal hydrocarbon degradation in a hypersaline environment

Shayantan Mukherji[1] and Abhrajyoti Ghosh[1,]✉

[1]*Bose Institute, Department of Biochemistry, P-1/12 CIT Scheme VIIM, Kolkata, India;*
✉***Corresponding author, E-mail: abhrajyoti.ghosh@jcbose.ac.in***

Abstract

Sundarban is the largest single stretch of halophytic coastal mangroves in the world. In recent years, several metagenomic studies have been performed which aimed at revealing the community structure of the resident microbial population of these saline coastal forests. The results revealed a striking difference in the distribution pattern of the resident archaeal population, with an increased population of Haloarchaea in regions with traditional histories of hydrocarbon/oil pollution. However, no studies have yet been performed to understand the active fraction of the resident microbial population of these mangroves. Thus, our study focused on both understanding the active fraction of the resident archaeal population of Sundarban along with an attempt at isolation and characterization of hydrocarbon degrading haloarchaea from these mangrove sediments. A total of 11 haloarchaeal isolates showed promise in their abilities to survive in presence of pHBA. A detailed biochemical characterization followed along with preliminary studies into their abilities to survive in presence of different hydrocarbons. The 11 isolates showed varying abilities to survive in presence of four different hydrocarbons in both rich and minimal media. HPLC analysis of 4 of the most potent strains when grown in pHBA showed a considerable percentage of decrease in hydrocarbon concentration over time. Trials using these 4 isolates to reduce the COD of polluted water of Sundarban regions also showed positive results. Thus, overall, these results show promise in the possible use of these haloarchaeal isolates as possible bioremediation agents especially in regions under high saline stress such as the Sundarban mangroves.

Keywords: haloarchaea, HPLC, hydrocarbon degradation, mangroves.

Acknowledgements. This work was supported by funds from CSIR, Government of India.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 102)

'Red – the magic color for solar salt production' – but since when?

Aharon Oren[1,✉] and Fan-Wei Meng[2]

[1]The Hebrew University of Jerusalem, Jerusalem 9190401, Israel; [2]Nanjing Institute of Geology and Paleaontology, Chinese Academy of Sciences, Nanjing 210008, China;
*✉**Corresponding author, E-mail: aharon.oren@mail.huji.ac.il.***

Abstract

'*Red – the magic color for solar salt production*' (Litchfield, 1991) refers to the characteristic color of saltern crystallizer ponds, due to dense communities of carotenoid-rich *Halobacteria* (Archaea), the bacterium *Salinibacter* and the alga *Dunaliella*. However, the first report from the western world mentioning red brines in salt production facilities probably dates from 1765: the *Encyclopédie* of Diderot. Earlier descriptions of marine solar salterns since Roman times do not mention red colors of crystallizer brines. These include the *Astronomica* of Manilius, *Naturalis Historia* by Pliny (both 1st century), the description of salterns near Volterra in *De Reditu Suo* by Namatianus (5th century), Agricola's *De Re Metallica* (1556) and an anonymous detailed description of French salterns (1669). Based on this negative evidence, we hypothesize that in earlier times saltern brines were never red. In salterns operated today in the traditional way as practiced in the Middle Ages, no red brines are observed. Crystallizer brines in the salterns of Sečovlje (Slovenia) and Ston (Croatia) contain up to $3x10^6$ prokaryotic cells/ml only, an order of magnitude lower than the brines of modern salt production facilities. We attribute the much lower microbial densities in traditional salterns to the much shorter brine residence time. In China, red saltern brines were documented earlier: in Li Shizhen's compendium of Materia Medica *Ben Cao Kang Mu*, completed in 1578 but based on older sources.

Keywords: history; archaea; carotenoids; pigmentation; salterns.

Acknowledgements. This work was supported by grants from the Israel Science Foundation (Grant No. 2221/15), the National Natural Science Foundation of China (Project No. 4151101015) and the Natural Science Foundation of Jiangsu Province (Project No. BK20151611).

References

Litchfield, C.D. (1991). Red – the magic color for solar salt production. In: Hocquet J. C., Palme R. (eds.). *Das Salz in der Rechts- und Handelsgeschichte.* Berenkamp, pp. 403-412. Schwaz, Austria.

Baas-Becking, L.G.M. (1931). Historical notes on salt and salt-manufacture. *Sci Month* **32**: 434-446.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 103)

Plant growth promoting potential of moderate halophilic coastal sand dune isolate *Bacillus marisflavi* strain K7SpZMAO002 of Goa - India

Sulochana Shet[1,✉] and Sandeep Garg[1]

[1] *Department of Microbiology, Goa University, Taleigao Plateau- 403206, Goa-India;*
✉ ***Corresponding author, E-mail: micro.sulochana@unigoa.ac.in***

Abstract

Bacillus marisflavi has the ability to grow at extreme conditions such as high salt, temperature and pH. In the present study, we isolated *Bacillus marisflavi* K7SpZMAO002 from the rhizhosphere of *Spinifex littoreus*, a Coastal Sand Dune (CSD) pioneer grass present at the foredune of Keri beach, Goa-India. The foredune of CSD is constantly affected by salt spray and is also under constant tidal effect, due to which it is a potential source of halophilic bacteria. During this study it was discovered that *Bacillus marisflavi* K7SpZMAO002 exhibits potential plant growth promoting attributes such as solubilization of inorganic phosphate at alkaline pH, production of indole-3-acetic acid, siderophore, exopolysaccharide (EPS), 1-aminocyclopropane-1-carboxylate (ACC) deaminase and antifungal activity against phytopathogens. Seed germination and seedling development of cowpeas (*Vigna unguiculata*) bacterized with *Bacillus marisflavi* K7SpZMAO002 exhibited 30% higher vigor index than the control seeds without inoculum. The i*n-vitro* studies of bio-inoculum on cowpea seeds in salt-affected coastal sand revealed 45% and 22% higher vigor index than negative (without inoculum) and positive (commercial biofertilizer) control, respectively. *Bacillus marisflavi* has the potential of increasing the growth of plants under the saline conditions and could be successfully used as biofertilizer in areas of high salinity where their non halophilic counterparts used in biofertilizer formulations might not be effective.

Keywords: *Bacillus marisflavi*, biofertilizer, coastal sand dune.

Acknowledgements. This work was suported by a UGC National Fellowship for an OBC student.

Tat-filaments – novel type of archaeal surface structures

Anna Galeva[1,✉], Alexey Syutkin[1], Mikhail Pyatibratov[1], Igor Kireev[2] and Oleg Fedorov[1]

[1]Institute of Protein Research, Russian Academy of Sciences, Institutskaya str. 4, Pushchino, Moscow region, 142290, Russia; [2]A.N. Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow 119991, Russia;
✉***Corresponding author, E-mail: galevanya@gmail.com.***

Abstract

Here we describe Tat-filaments – a completely new kind of archaeal surface appendages isolated from haloarchaeon *Haloarcula hispanica*. The uniqueness of these structures is that they are formed of a protein subunit secreted using the Tat-pathway in contrast to all known archaeal surface structures secreted by the Sec-pathway. We isolated surface structures from *H. hispanica* (by PEG-mediated precipitation) and using electron microscopy we found along with archaella (10-11 nm in diameter) thinner filaments with a diameter of ~ 3 nm. On the SDS-electropherogram we observed an additional major protein band along with the archaellins. An increase in salinity of the growth medium results in an increase of the relative content of this protein and it correlates with an expansion in the number of thin filaments relative to archaella in the preparations. Mass spectrometry revealed that this extra protein band corresponds to the product of the *hah_0240* gene, which encodes, according to the annotation, a "hypothetical protein". Bioinformatic analysis showed that the HAH_0240 protein contains a twin-arginine motif used in the so-called twin-arginine translocation (Tat) pathway, a special secretion way for proteins in plants, Bacteria and Archaea. However, no evidence of the use of the Tat-pathway in the assembly of filamentary structures in Archaea has been described to this moment. The found filaments ("Tat-filaments") are the first type of such structures. The role of the Tat-filaments still remains elusive, but our preliminary data suggest that these structures may be involved in biofilm formation and in cell adhesion to surfaces.

Keywords: archaeal surface structures, *Haloarcula hispanica*, Tat-filaments, Tat-pathway.

Acknowledgements. This study was supported by RFBR (grant № 19-04-01327 A) and the MSU Development Program (PNR 5.13).

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 105)

Comparative studies of *Halorubrum lacusprofundi* archaella synthesized in different ways

Anna Galeva[1], Mikhail Pyatibratov[1,✉], Alexey Syutkin[1], Tessa E.F. Quax[2], Tatjana Melnik[1], Alexey Surin[1], Sergei Beznosov[1] and Oleg Fedorov[1]

[1]Institute of Protein Research, Russian Academy of Sciences, Institutskaya st., 4, Pushchino, Moscow Region, 142290, Russia; [2]University of Freiburg, Institute for Biology II- Microbiology, Archaeal virus-host interactions, Schänzlestrasse 1, 79104 Freiburg, Germany;
✉Corresponding author, E-mail: bratov@vega.protres.ru.

Abstract

The role of multiplicity of archaellin genes in Euryarchaea remains mysterious. The haloarchaeon *Halorubrum lacusprofundi* DL18 possesses an operon with two archaellin genes encoding the extraordinarily diverged paralogs FlaB1 and FlaB2. In the archaellar filaments both proteins are represented in approximately equimolar amounts. We compared DL18 with another strain ACAM34 with only one *flaB2* gene. Both strains synthesize functional archaella, but motility is better in DL18. Using heterologous expression in a *Haloferax volcanii* strain lacking its own archaellin genes, we obtained three recombinant filament types: B1, B2 and B1B2. All archaellin-expressing *Hfx. volcanii* strains were motile, indicating the recombinant archaella functionality. Like the natural *Hrr. lacusprofundi* strains, the motility of cells with B2-filaments was significantly lower than for cells synthesizing B1B2- and B1-archaella. Electron microscopy of native and recombinant filaments showed that the B2-archaella are quite flexible and often twisted in loops and tangles, while the B1B2- and B1-archaella look like more typical. Both native and recombinant B1B2-filaments have greater thermal stability and resistance to low salinity stress than single-component filaments. The data obtained indicate a close interaction between the FlaB1 and FlaB2 in the filament structure. Thus, the two-component composition is needed for additional stabilization of the archaellum structure and adaptation to a wider range of external conditions and is not necessary for archaella supercoiling.

Keywords: archaella, archaellins, *Halorubrum lacusprofundi*, motility.

Acknowledgements. The work was supported by the RFBR grant No. 19-04-00613 A.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 106)

Analysis of genes that mediate persistence in halophilic microbes subjected to osmotic shock

Margot R. Miranda-Katz[1], Abby M. Gregory[1], Serena M. Graham[1] and Ronald F. Peck[1,✉]

[1]*Colby College, 8800 Mayflower Hill, Waterville, Maine, USA;*
✉***Corresponding author, E-mail: ronald.peck@colby.edu.***

Abstract
All organisms are subject to stress from environmental changes, and understanding the specific molecular responses to allow cells to maintain viability is a fundamental biological problem. We explore this process in the halophilic microbe *Haloferax volcanii*. We hypothesize that they have evolved so that a subset of their population consists of cells prepared to survive dramatic stress events. As a first step to examine how these 'persisting' cells endure, we used RNA sequencing to examine transcriptome differences in cells that survived osmotic shock compared to unstressed cells. Our data revealed that approximately half of the protein-coding genes exhibited statistically significant differences in transcriptome abundance with a nearly equal distribution of more abundant and less abundant transcripts. There were about twice the number of transcripts exhibiting a 10-fold or greater decrease (5.3% of protein-coding genes) in abundance than those with the same magnitude of increased abundance (2.7% of protein-coding genes). These results may indicate that cells primed for survival reduce production of proteins required for active metabolism. We are currently conducting experiments to determine if deletion or overexpression of some of these genes affects susceptibility of *H. volcanii* to osmotic shock. Our work may provide insight into why some cells in a microbial population survive stress events despite being genetically identical to cells that are killed.

Keywords: gene regulation, heat shock proteins, universal stress proteins.

Acknowledgements. This project was supported by an Investigator Award to R.F.P from an Institutional Development Award from the National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health under grant P20GM103423. Additional funding was provided by the Colby College Natural Science Division.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 107)

Analysis of the expression and function of *Haloferax mediterranei lsm1* gene

Gloria Payá[1], Vanesa Bautista[1], Mónica Camacho[1], Julia Esclapez[1] and María José Bonete[1,✉]

[1]University of Alicante, Agrochemistry and Biochemistry Department, Ap 99, E-03080 Alicante, Spain; ✉***Corresponding author, E-mail: mjbonete@ua.es***

Abstract

Proteins of Lsm family are key players in RNA metabolism. However, archaeal homologs of these proteins (Like-Sm proteins) have been less studied than in the rest of the domains of life. *H. mediterranei* encodes a single *lsm* gene, which overlaps by four nucleotides with the *rl37e* gene. The gene environment anaylsis has revealed that it is highly conserved in *Archaea*. In most of the archaeal genomes analysed, the *rpl37e* gene follows the *lsm* gene. In 56 genomes, the two genes overlap and in 21 genomes they are widely spaced, so the cotranscription could be assumed. Expression and co-transcription with the *rl37e* gene were analysed by RT-PCR in complex medium, defined medium with nitrate or ammonium as nitrogen source, and nitrogen starvation at different times. The *lsm* and *rl37e* genes are expressed in all conditions, but only cotranscribe under nitrogen starvation conditions. Gene expression microarray shows that the *lsm* gene presents non-significant expression differences in different nitrogen sources. To determine the Lsm function, deletion mutants of *lsm1* gene (HM26-Δ*lsm*) and *Sm1* domain (HM26-Δ*Sm1*) were generated. These mutants were characterised in the presence of various sources of nitrogen and complex medium. Moreover, swarming assays were carried out and the cell morphology was analysed in the different growth phases in the same culture media. Finally, heat shock, oxidative stress and ethanol stress assays were performed. The deletion of the *lsm* gene and Sm1 domain are viable, resulting in a pleiotropic phenotype, indicating that the haloarchaeal Lsm protein is involved in many cellular processes.

Keywords: deletion mutants, haloarchaea, Lsm protein, microarray, RT-PCR.

Acknowledgements. This work was suported by BIO2013-42921-P. Gloria Payá enjoys a grant ACIF/2018/200.

Transcriptional regulators of *Haloferax mediterranei* related to nitrogen source

Gloria Payá[1], Vanesa Bautista[1], Mónica Camacho[1], Julia Esclapez[1] and María José Bonete[1,✉]

[1]University of Alicante, Agrochemistry and Biochemistry Department, Ap 99, E-03080 Alicante, Spain;
✉Corresponding author, E-mail: mjbonete@ua.es

Abstract

The haloarchaeon *Haloferax mediterranei* is able to grow in the presence of different inorganic and organic nitrogen sources under aerobic conditions. In order to identify genes with potential importance in nitrogen assimilation and its regulation mechanisms, the global gene expression was analysed in different nitrogen sources, such as ammonium, nitrate and nitrogen starvation, by microarray technology. The results indicate that the absence of ammonium is the responsible factor for the expression of genes involved in nitrate assimilation pathway. Moreover, seven transcriptional regulators with significant differential expression have been identified: transcriptional regulator of ArsR family (*arsR1*, HFX_RS03970 and *arsR2*, HFX_RS03540), PadR family (*padR,* HFX_RS03340), and AsnC family (*lrp,* HFX_RS12185); GvpE transcriptional regulator (*gvpE,* HFX_RS08310); *wecE* pleiotropic regulatory protein (*wecE,* HFX_RS17500) and hypothetical regulator (HFX_RS17105). The *gvpE* gene and hypothetical regulator are up-expressed in nitrogen starvation and nitrate respect to ammonium, while *arsR1* is down-expressed in the same conditions. The *padR* gene is down-expressed and the *lrp* gene is up-expressed in nitrogen starvation respect to ammonium. On the other hand, the *arsR2* gene is up-expressed and the *wecE* is down-expressed in nitrate respect to ammonium. Microarray data were validated by qRT-PCR. To clarify the function of these seven regulators in the nitrogen and amino acids metabolisms, a meticulous bioinformatic analysis was carried out. This work represents a study of transcriptional profiles related to nitrogen assimilation and amino acids metabolism in extreme halophilic microorganisms, which allows the identification of transcriptional regulators.

Keywords: haloarchaea, microarray, qRT-PCR, transcriptional regulator.

Acknowledgements. This work was suported by BIO2013-42921-P. Gloria Payá enjoys a grant ACIF/2018/200.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 109)

sRNAome in response to nitrogen source of *Haloferax mediterranei*

Gloria Payá[1], Vanesa Bautista[1], Mónica Camacho[1], María José Bonete[1] and Julia Esclapez[1,✉]

[1]University of Alicante, Agrochemistry and Biochemistry Department, Ap 99, E-03080 Alicante, Spain;
✉Corresponding author, E-mail: julia.esclapez@ua.es

Abstract

In *Archaea* domain, the knowledge about small RNAs (sRNAs) and the physiological function they carry out has been studied in less detail than in the rest of life domains. RNA-Seq has been performed in different nitrogen sources, 40 mM nitrate and 40 mM ammonium, to identify the sRNAs expressed in both conditions and understand the regulatory networks involved in the transcriptional regulation of nitrogen assimilation metabolism in *H. mediterranei* ATCC33500. In total, 460 sRNAs were identified manually by using the high-performance visualisation tool, Integrative Genomics Viewer (IGV). Significant differences were observed in the expression pattern of 102 sRNAs according to the nitrogen source after analysis of the RNA-Seq data differential expression. Moreover, the secondary structure of these sRNAs, their possible gene targets and the sequence conservation in other archaeas have been determined. In addition, the molecular function, cellular component and biological process of target genes were analysed based on nitrogen source. As result, sRNAs which show differences in their expression patterns, could be directly related to proteins involved in nitrogen assimilation pathways, such as glutamate dehydrogenase (sRNA311 and sRNA276), glutamine synthetase (sRNA274 and sRNA310), the nitrogen regulatory PII protein (sRNA228) and nitrate/nitrite transporter (sRNA451). Curiously, the sRNA87 could regulate the nitrous oxide reductase accessory protein NosL, which is involved in denitrification. Finally, 10 selected sRNAs were validated by RT-PCR. These results suggest that these sRNAs could be involved in the regulation of nitrogen assimilation and can be implicated in important gene regulatory mechanisms.

Keywords: gene regulation, haloarchaea, nitrogen metabolism, RNA-Seq, small RNAs (sRNAs).

Acknowledgements. This work was suported by BIO2013-42921-P. Gloria Payá enjoys a grant ACIF/2018/200.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 110)

Making the stress-protectant ectoine: Structural and biochemical insights into the acetyltransferase EctA

Alexandra A. Richter[1], Stefanie Kobus[2], Laura Czech[1], Astrid Höppner[2], Jan Zarzycki[3], Tobias Erb[3,4], Lukas Lauterbach[5], Jeroen S. Dickschat[5], Erhard Bremer[1,4,✉] and Sander H.J. Smits[6,✉]

[1]Department of Biology, Laboratory for Microbiology, Philipps-University Marburg, D-35043 Marburg, Germany; [2]X-ray Facility and Crystal Farm, Heinrich-Heine-University Düsseldorf, D-40225 Düsseldorf, Germany; [3]Max-Planck-Institute for Terrestrial Microbiology, D-35043 Marburg, Germany; [4]LOEWE Center for Synthetic Microbiology, Philipps-University Marburg, D-35043 Marburg, Germany; [5]Kekulé-Institute for Organic Chemistry and Biochemistry, Friedrich-Wilhelms-University Bonn, D-53121 Bonn, Germany; [6]Institute of Biochemistry, Heinrich-Heine-University Düsseldorf, D-40225 Düsseldorf, Germany; ✉***Corresponding co-authors, E-mail: sander.smits@hhu.de, bremer@staff.uni-marburg.de.***

Abstract

Ectoine is a compatible solute widely synthesized by microorganisms as an osmostress protectant. Due to its stabilizing effect on macromolecules, it is adressed in the literature as a chemical chaperone and used for medical and biotechnological applications. The 2,4-diaminobutyrate acetyltransferase (EctA) catalyzes the second step of the ectoine synthesis pathway, the regio-specific acetylation of 2,4-diaminobutyrate (DAB) to N-γ-acetyl-2,4-diaminobutyric acid (ADABA). Here we focus on the biochemical and structural characteristics of EctA from the thermo-tolerant bacterium *Paenibacillus lautus*. A codon-optimized *ectA* gene of *P. lautus* was cloned into expression vectors for heterologous expression in *Escherichia coli*. The produced protein was purified, used for enzymatic assays, and crystallized. The enzyme activity of EctA was assessed by photometry-based assays measuring the formation of the co-product CoA. Biochemical characterization of the wildtype enzyme showed optimal activity conditions at high temperature (>40 °C) and high pH-values (8.5-9.5). Crystal structures of apo-EctA, and EctA in complex with the substrate DAB and CoA were obtained. These revealed a functional dimer and illuminated the architecture of the enzymes active site. Putative DAB-binding amino acids were verified by site-directed mutagenesis studies.

Keywords: acetyltransferase, compatible solute, crystal structure, ectoine, osmostress protection.

References

Czech, L., Hermann, L., Stöveken, N., Richter, A.A., Höppner, A., Smits, S.H.J., Heider, J., & Bremer, E. (2018). Role of the extremolytes ectoine and hydroxyectoine as stress protectants and nutrients: Genetics, phylogenomics, biochemistry, and structural analysis. *Genes*. **9**, 177.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 111-112)

Transcriptional analysis of *glnA* deletion mutant in *Haloferax mediterranei*

Verónica Rodríguez-Herrero[1], Vanesa Bautista[1], Mónica Camacho[1], Mónica Cortés[2], María-José Bonete[1] and Julia Esclapez[1,✉]

[1]University of Alicante. Science Faculty. Biochemistry and Molecular Biology, Carretera San Vicente del Raspeig s/n, Alicante, Spain; [2]University of Alicante. Polytechnic School. Applied Mathematics, Carretera San Vicente del Raspeig s/n, Alicante, Spain; ✉***Corresponding author, E-mail: julia.esclapez@ua.es***

Abstract

Haloferax mediterranei (ATCC 33500T) genome contains three genes which present sequence homology with glutamine synthetase: *glnA, glnA2* and *glnA3*. Deletion mutants of *glnA* gene were constructed to determine the role of this enzyme in the nitrogen and amino acid metabolism. The mutant HM26-Δ*glnA* has been characterised using different approaches, concluding that *glnA* is an essential gene to the growth of *H. mediterranei*. The transcriptomes of deletion mutant HM26-Δ*glnA* and parental strain (HM26) have been compared in different growth conditions. Statistical analysis of the global gene expression was carried out obtaining different expression profiles depending on the contrast analysed, showing up and down-regulated genes. In addition, a functional gene analysis was performed to find out the processes in which they are involved. The microarray data was confirmed by quantitative real-time PCR. The transcriptomes comparison results of HM26-Δ*glnA* against HM26 in the presence of nitrogen source shows that 3 genes are up -regulated and 50 are down-regulated. The great majority of down-regulated genes are related to amino acid metabolism and the transport system. However, the up-regulated genes were related to sulphur metabolism. The analysis of the data in the nitrogen absence shows changes in the transcription level of great number of genes in both HM26-Δ*glnA* and HM26. In this condition, the mutant shows 91 up-regulated and 79 down-regulated exclusive genes, which are mostly related to nitrogen metabolism compounds, transporters and regulation processes. These results suggest that the HM26-Δ*glnA* uses different metabolic strategies from that of the parental strain under nitrogen starvation conditions.

Keywords: deletion mutant, glutamine synthetase, microarray, nitrogen metabolism.

Acknowledgements. This work was supported by Project BIO2013–42921–P by MINECO and co-financed by the European Regional Development Fund (FEDER).

References

Pedro-Roig, L., Camacho, M. & Bonete, M.J. (2013). Regulation of ammonium assimilation in *Haloferax mediterranei*: Interaction between glutamine synthetase and two GlnK proteins. *Biochim Biophys Acta* **1834**: 16–23.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 113)

Catabolism of the extremolytes ectoine and hydroxyectoine – Regulation and mechanisms

Lucas Hermann[1,✉] and Erhard Bremer[1,✉]

[1] *Philipps-Universität Marburg, Laboratory for Microbiology, Karl-von-Frisch-Str. 8, 35043 Marburg, GER;* ✉***Corresponding authors, E-mail: lucas.hermann@biologie.uni-marburg.de, bremer@staff.uni-marburg.de.***

Abstract

Ectoine and its derivative hydroxyectoine are widely used as osmostress protectants by members of the *Bacteria*. A large group of *Proteobacteria* can metabolize these osmolytes and use them as sole carbon, nitrogen and energy sources. In the marine *Roseobacter* species *Ruegeria pomeroyi* the ectoine utilization operon consists of the core metabolic enzymes EutABC which seem to be involved in the degradation of hydroxyectoine and EutDE which catabolize ectoine. EutD opens the pyrimidine ring of ectoine to form N-α-ADABA and EutE deacetylates this intermediate to DAB which then can fuel the cells energy metabolism. The operon is tightly regulated by the GntR-type protein EnuR which binds to two specific operator sites upstream of the ectoine/ hydroxyectoine importer and catabolic gene cluster. An EnuR-K302H mutant and reduction by $NaBH_4$ show that PLP is covalently bound by EnuR and is used to detect N-α-ADABA and DAB as inducers. This changes the DNA-binding affinity of EnuR, which is shown by EMSAs. Bioinformatic analysis suggests the widespread use and adaptations of this metabolic pathway, and therefore implicates ectoine metabolisms in global carbon and nitrogen cycles driven by bacteria.

Keywords: ectoine, GntR, hydroxyectoine, metabolism, regulation.

Acknowledgements. This work was supported by the German Research foundation (SFB 987 " Microbial diversity in environmental signal response").

References

Schulz, A., Hermann, L., Freibert, S.A., Bönig, T., Hoffmann, T., Riclea, R., Dickschat, J.S. Heider, J., & Bremer, E. (2017). Transcriptional regulation of ectoine catabolism in response to multiple metabolic and environmnetal cues. *Environ. Microbiol.* **19**, 4599–4619.

Czech, L., Hermann, L., Stöveken, N., Richter, A.A., Höppner, A., Smits, S.H.J., Heider, J., & Bremer, E. (2018). Role of the extremolytes ectoine and hydroxyectoine as stress protectants and nutrients: Genetics, phylogenomics, biochemistry, and structural analysis. *Genes*. **9**, 177.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 114)

Reassessment of the taxonomic position of the genera *Haloterrigena* and *Natrinema*

Hiroaki Minegishi[1,2,✉], Yasuhiro Shimane[2], Masahiro Kamekura[3], Antonio Ventosa[4] and Takashi Itoh[5]

[1]*Toyo University, 2100 Kujirai, Kawagoe-shi, Saitama, Japan;* [2]*Japan Agency for Marine-Earth Science and Technology, 2-15 Natsushima-cho, Yokosuka-shi, Kanagawa, Japan;* [3]*Halophiles Research Institute, 677-1 Shimizu, Noda-shi, Chiba, Japan;* [4]*University of Sevilla, 41012 Sevilla, Spain;* [5]*RIKEN BioResource Center, 3-1-1 Koyadai, Tsukuba-shi, Ibaraki, Japan;*
✉***Corresponding author, E-mail: minehiro@toyo.jp.***

Abstract

The genus *Natrinema* was created in 1998 to accommodate some misclassified halobacterial strains, while the genus *Haloterrigena* was established in the following year. Since then, it has been known that the two genera overlap to some extent in phylogenetic trees based on 16S rRNA gene sequences. The numbers of species of genera *Haloterrigena* and *Natrinema* increased to six and five, respectively by 2019. We suggested that the two genera might constitute a single genus based on studies of full-length gene sequences of the RNA polymerase subunit B', as well as 16S rRNA gene sequences. Recently, Ding *et al*. (2017) and Rasooli *et al*. (2017) showed that species of the genera *Natrinema* (7 species) and *Haloterrigena* (10 species) formed a monophyletic group in 16S rRNA gene sequence trees generated using neighbour-joining and maximum likelihood algorithms. Reclassification of species presently belonging to the genus *Haloterrigena* as *Natrinema* species may be justified in the near future. In this study, the relatedness of 17 species belonging to the genera *Haloterrigena* and *Natrinema* was investigated through phylogenetic analysis (by the use of 16S rRNA and *rpoB'* genes) and the assessment of the average nucleotide blast identity (ANI) and the percentage of conserved proteins (POCP). Results of ANI vs POCP and 16S rRNA gene vs POCP with 17 species in the genera *Haloterrigena* and *Natrinema*, confirmed that these strains are very likely to constitute a monophyletic group.

Keywords: 16S rRNA gene, ANI, *Haloterrigena*, *Natrinema*, POCP

References

Ding, J. Y., Chen, S. C., Lai, M. C., & Liao, T. L. (2017). *Haloterrigena mahii* sp. nov., an extremely halophilic archaeon from a solar saltern. *Int J Syst Evol Microbiol* **67**(5):1333-1338.

Raasooli, N., Naghoni, A., Amoozegar, M.A., Mirfeizi, L., Moshtaghi N. M., Shahzadeh Fazeli *et al.* (2017). *Natrinema soli* sp. nov., a novel halophilic archaeon isolated from a hypersaline wetland. I*nt J Syst Evol Microbiol* **67**(7):2142-2147.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 115)

Microbial interactions in the Dead Sea as reflected by enrichment cultures: the curious case of Nanohaloarchaea

Uri Neri[1], Yutian Feng[2], J. Peter Gogarten[2] and Uri Gophna[1]

[1]Tel Aviv University, Tel Aviv 69978, Israel; [2]Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269, USA; ✉***Corresponding author, E-mail: urigo@tau.ac.il***

Abstract

Since their initial discovery in late 2011, the lifestyle of Nanohaloarchaea has been shrouded in mystery, leading to an ongoing debate regarding their host dependence, and placement on the evolutionary tree of life. Recently we carried out a survey of the viral and microbial communities from current day Dead Sea, and combined direct visualization by TEM with metagenomic sequencing. Our survey revealed a large variety of viruses and hosts that still persist in the environment, despite the gradual evaporation of the dying Dead Sea. Strikingly, when we fractionated by filter size we observed a large morphological variety of viruses and small cells. We then continued to investigate these intriguing entities by serially propagating the environmental microbial seed in several distinct enriched growth media. By comparing the different microbial communities (via both TEM and metagenomic sequencing) between different media and along culture and time points, we were able to determine multiple interactions between both the viruses and their hosts, as well as between the newly discovered small-sized cellular entities. We obtained an enrichment culture composed almost exclusively of a representative of the Nanohaloarchaea, and using metagenomics we were able to obtain the first complete closed circular genome of it, including an integrated provirus. Different enrichment cultures had different distinct species of Nanohaloarchaea, along with a number of completely novel viruses, expanding the known haloarchaeal - viral repertoire.

Keywords: computational genomics, halophilic archaea, horizontal gene transfer, host, metagenomics, virus.

Acknowledgements. This work was supported by the BSF grant (2016671), VW foundation grant Experiment! (Discovering RNA Viruses of Archaea) and ERC AdG (CRISPR-EVOL).

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 116)

Mechanistic insights into cell fusion in *Haloferax*

Yarden Shalev[1], Jerry Eichler[2] and Uri Gophna[1,✉]

[1]Tel Aviv University, Tel Aviv 69978, Israel; [2]Ben Gurion University of the Negev, Beersheva 8410501, Israel; ✉***Corresponding author, E-mail: urigo@tau.ac.il.***

Abstract

The intriguing phenomenon of lateral gene transfer mediated by cell fusion in halophilic archaea, also known as mating, has been recognized since the 1980s; however, the molecular mechanisms involved remain largely unknown. Within the *Haloferax* genus, both the Surface-layer (S-layer) protein and the glycans that decorate it, vary between species, which can potentially result in many different surface types, analogous to bacterial serotypes. This variation may mediate important phenotypes, such as sensitivity to different viruses as well as mating preferences. We have previously shown how differences in *H. volcanii* surface glycosylation influence cell fusion and how environmental and genetic perturbations to this process can dramatically affect fusion efficiency. These findings suggest that surface glycosylation may play a role in cellular recognition and within-species mating preferences in halophilic archaea, thereby affecting gene exchange and speciation processes. Our main objectives are to investigate the mating mechanism in haloarchaea and determine the effect of cell surface *N*-linked glycan composition in the genus *Haloferax* on cell fusion. To that end, we have recently established a new fluorescent marker-based protocol for rapid and more accurate quantification of mating efficiency that is independent of selectable genetic markers. Using both genetic and fluorescence-based approaches we will attempt to further establish the effect of S-layer glycan composition on the first step of mating, cell-cell recognition.

Keywords: glycosylation, halophilic archaea, horizontal gene transfer, mating, S-layer

Acknowledgements. This work was supported by the BSF grant (2016671) and an ERC grant (CRISPR-EVOL).

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 117)

Biosynthesis of selenium nanoparticles by *Haloferax alexandrinus* GUSF-1 (KF796625) and their use in modulating the size and shape of calcium oxalate crystals

Jyothi J. Alvares[1] and Irene J. Furtado[1,]✉

[1]*Goa University, Faculty of Life Sciences and Environment, Department of Microbiology, Taleigao Plateau, Goa- India, 403206;* ✉***Corresponding author, E-mail: ijfurtado@unigoa.ac.in***

Abstract

The ability to resist and biotransform toxic metalloids and chalcogens though widespread among prokaryotes is relativley less explored among the archaeal domain (Srivastava and Kowshik, 2013). In this study, a haloarchaeon, *Haloferax alexandrinus* GUSF-1 (KF796625) isolated from solar saltern of Goa – India grew optimally at 25% NaCl and was resistant to 120 mM concentration of toxic selenite (SeO_3^{2-}), with formation of deep red particulate matter in 4 days. Whole cells incubated with SeO_3^{2-} also gave the red particulate matter. SEM-EDX of the red material exhibited absorption peaks at 1.5, 11.2, and 12.5 keV thus indicating the presence of selenium. Further, the 8 diffraction peaks between 23 and 62 θ in XRD, corresponding to planes at (100), (101), (110), (012), (111), (201), (112) and (022) matched with those for selenium (Se^0) in JCPDS card No. 073-0465. TEM micrograph revealed rhombic shaped nanoparticles with capping material. The SeNPs at 100 µg/mL were able to substantially modulate the size and hexagonal shape of the calcium oxalate crystals to smooth spheres. Conclusively, this study gives an important lead for harnessing the potential of *Haloferax alexandrinus* GUSF-1 (KF796625) for biogenic synthesis of Se^0 from SeO_3^{2-} and for exploring the possible use of Se^0 in pharmaceutical application.

Keywords: calcium oxalate crystals, haloarchaeon, *Haloferax*, selenium nanoparticles

References:

Srivastava, P., & Kowshik, M., (2013). Mechanisms of metal resistance and homeostasis in haloarchaea. *Archaea:* 1–16.

Haloarchaeal strains as a new source for anti-cancer natural components

Mohammad Ali Amoozegar[1,✉] Atefeh Safarpour[1] and
Seyed Abolhassan Shahzadeh Fazeli[2,3]

[1]Extremophiles Laboratory, Department of Microbiology, School of Biology, College of Science, University of Tehran, Tehran, Iran; [2]Microorganisms Bank, Iranian Biological Resource Centre (IBRC), ACECR, Tehran, Iran; [3]Department of Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, ACECR, Tehran, Iran; ***✉Corresponding author, E-mail: amoozegar@ut.ac.ir***

Abstract

Their ability to live in salt concentrations makes halophiles a good choice for a search for novel bioactive natural components with different medicinal applications. The role of metabolites from haloarchaeal strains belonging to different groups for application in cancer treatment is not well studied (Hou & Cui, 2018). A total of eight haloarchaeal metabolites have been evaluated for their anticancer potential against five human cancer cell lines of breast, lung and prostate cancers, while using a fibroblast cell line as a control. Downstream analysis including sphere and colony formation assay, invasion and migration assays, real-time PCR analysis, cell cycle, apoptosis assays and *in vivo* experiments were done to find the most potent metabolite with efficient anti-cancer activity. All archaeal metabolites investigated were found to be cytotoxic against one or more cancer cell lines and/or normal cells after 48 h with cell line specific activity. A metabolite from one strain has a potent cytotoxic effect on Du-145 and PC3 cell lines with an IC50 of 500 μg/ml without any effects on normal cells, and reduced sphere and colony formation ability of DU145 and PC3 cells with down-regulation of *SOX2* gene expression. This metabolite affected the invasion and migration ability of these cell lines. It significantly increased both early and late apoptosis in the androgen-dependent PC3 cell line and caused tumor shrinkage in human prostate tumors in nude mice. The results of the present study indicated a major anti-cancer effect of metabolites derived from haloarchaeal strains.

Keywords: cancer, haloarchaea, sphere formation.

References

Hou, J., & Cui, HL. (2018). *In-vitro* antioxidant, antihemolytic, and anticancer activity of the carotenoids from halophilic archaea. *Curr Microbiol* **75**: 266-71.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 119)

Halotolerant Black Sea bacteria for bioplastics production

Iulia Chiciudean[1,✉], Ioana Mereuţă[1], Elena Luiza Mihalachi[1], Irina Lascu[1], Sorin Marius Avramescu[2], Piotr Jablonski[3], Mikkel Christensen[4], Hilde Hansen[4], Ileana Stoica[1] and Ana-Maria Tănase[1]

*[1]Department of Genetics, Faculty of Biology, University of Bucharest, Bucharest, Romania; [2]Faculty of Chemistry, University of Bucharest, Bucharest, Romania; [3]Department of Chemistry, Faculty of Science and Technology, Umeå University, Umeå, Sweden; [4]The Norwegian Structural Biology Centre, University of Tromsø, Tromsø, Norway; ✉**Corresponding author, E-mail: iulia.chiciudean@bio.unibuc.ro.***

Abstract

To address plastic pollution environmental concerns, it is of high importance to develop novel types of biodegradable polymeric materials that can be sustainably and economically produced. One bioplastic class with a great potential of exploitation are polyhydroxyalkanoates (PHAs), natural polymers produced by bacteria, with properties similar to oil-derived polypropylene/polyesters. This study focuses on isolation and characterization of halotolerant bacteria able to produce high amounts of PHAs from low-cost biomass. From a collection of 200 Black Sea bacterial isolates, screened for PHAs production by Red Nile staining and epifluorescence microscopy, strain N16b, identified as *Halomonas* sp., presented high PHAs production on saline media (up to 20% NaCl) with sugar beet molasses as sole carbon source. GC-MS analysis confirmed that PHAs produced by strain N16b represent more than 50% of the cell mass and were mainly represented by 3-hydroxybutyrate and 3-hydroxyvalerate. The effect of osmolytes and pH on bacterial growth was tested using Biolog Phenotype MicroArrays. The presence of the PHA synthase (*phaC*) gene and ectoine synthase (*ectC*) gene, involved in the halotolerant phenotype, was confirmed after genome sequencing and annotation (Prokka). Thus, strain N16b a good candidate for bioplastic production from inexpensive industrial side product biomass (e.g. molasses), using salted water in the production process (an important feature for reducing the risk of contamination and for countries with limited fresh water resources).

Keywords: bioplastic, halotolerant bacteria, low-cost biomass polyhydroxyalkanoates.

Acknowledgements. This work was supported by the Romanian National Authority for Scientific Research and Innovation, CCCDI – UEFISCDI, project number 13/2017 ERANET-Marine Biotechnology MARPLAST, within PNCDI III.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 120)

The etiology of pink pigmentation on mural paintings. Case study: Humor Monastery, Romania

Roxana Cojoc[1,✉], Mădălin Enache[1], Simona Neagu [1], Gina Cogălniceanu[1], Monica Mitoi[1] and Ioana Gomoiu[1]

[1]*Institute of Biology Bucharest of the Romanian Academy, 296 Splaiul Independentei, Bucharest, Romania;* ✉***Corresponding author, E-mail: roxana.cojoc@ibiol.ro***

Abstract

The Humor Monastery located in Suceava County, Romania has been included since 1993 in the UNESCO heritage, as a cultural site. Due to the presence of efflorescences and improper microclimate conditions for conservation, pink pigmented areas showed on the wall surface, both in the prenave of the monastery and in the Burial Chamber, up to a height of 0,7 m from the ground. The aims of the present study were to establish the origin of pink pigmentation and to test colloidal solutions of gold and silver nanoparticles in order to establish a decontamination strategy for the affected areas and a treatment to prevent future colonisations. Moderately halophilic and halotolerant bacterial strains were isolated from samples of infilling mortar. They were morphologically characterised by optical microscopy and SEM, tested for salinity, pH and temperature optimal values for growth, enzyme synthesis, antibiotic susceptibility and capacity to form biofilms. The pigments produced by the isolated bacterial strains were extracted for FT-IR analysis, and some vegetal origin colloidal silver and gold nanoparticle solutions were tested for their inhibitory effect on the biodeteriogens growth. Based on the results obtained we suggest a bacterial origin of the pink discoloration responsible for the biodeterioration process in Humor Monastery. The efficiency of silver nanoparticles, obtained from white strawberry callus extract, in inhibiting the growth of carotenoid-producing halotolerant strains recommends them for decontamination treatment and for incorporation into infilling mortars used in restoration in order to prevent the biodeterioration process.

Keywords: biofilm, biopigmentation, halophiles, Humor Monastery

Acknowledgements. This research was financially supported by project no. RO1567-IBB05/2018 from the Institute of Biology Bucharest of Romanian Academy

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 121)

Stimulating effects of selenium and betaine on the halophyte microalga *Dunaliella salina*

Diana Constantinescu-Aruxandei[1], Alexandra Butean[1],
Alin Cristian Nicolae Vintilă[1], Alexandru Vlaicu[1], Ana-Maria Gălan[1],
Anca Paulenco[1], Sanda Velea[1] and Florin Oancea[1,✉]

*[1]National Institute for Research and Development in Chemistry and Petrochimistry - ICECHIM , Spl. Independenței nr. 202, sector 6, Bucharest; ✉**Corresponding author, E-mail: florin.oancea@icechim.ro.***

Abstract

Selenium (Se) stimulates accumulation of carotenoids in microalgae (Zheng *et al.,* 2017). Betaine acts as osmoprotectant for microalgae against salinity variation (Torstensson *et al.*, 2019). The aim of our study was to determine the effects of betaine and Se on growth of *Dunaliella salina* in media with 3% and 7% NaCl. A high throughput assay was done in 24- and 96 well-microplates, using 2 μM sodium selenite, 0.2 μM betaine and their combination. The microalgae growth was monitored by acquiring UV-VIS spectra between 400 and 800 nm. After 14 days of cultivation, the photosynthetic pigments were extracted by dodecane and quantified spectrophotometrically (Kleinegris, 2011). Both betaine and Se caused slight growth promotion and chlorophyll accumulation. Betaine significantly enhanced carotenoids accumulation, especially in medium with 7% salt. The mixture of betaine and Se had a similar effect. Both betaine and Se act as microalgae biostimulants, because they promote microalgae growth, increase microalgae tolerance to abiotic stress, including salt stress, and enhance the accumulation of photosynthetic pigments, carotenoids and chlorophyll. Optimization of this effect is important for higher carotenoids yields from *D. salina* cultivated non-axenically on media with high concentrations of salt.

Keywords: betaine, biostimulants, carotenoids, halophytes**,** selenium.

Acknowledgements. This work was suported by Ministry of Research and Inovation, Nucleu Programme, Project PN.19.23.01.01

References

Kleinegris, D. M., Janssen, M., Brandenburg, W. A., & Wijffels, R. H. (2011). Continuous production of carotenoids from *Dunaliella salina*. *Enzyme Microb Technol* **48**: 253-259.

Torstensson, A., Young, J. N., Carlson, L. T., Ingalls, A. E., & Deming, J. W. (2019). Use of exogenous glycine betaine and its precursor choline as osmoprotectants in Antarctic sea-ice diatoms. *J Phycol*. doi.org/10.1111/jpy.12839

Zheng, Y., Li, Z., Tao, M., Li, J., & Hu, Z. (2017). Effects of selenite on green microalga *Haematococcus pluvialis*: Bioaccumulation of selenium and enhancement of astaxanthin production. *Aquat Toxicol* **183**: 21-27.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 122)

Effects of nitrogen and phosphorus limitations on polyhydroxyalkanoate synthesis by *Halomonas elongata* strains grown under saline conditions

Adorján Cristea[1,✉], Ana-Maria Bratovianu[1], Andreea-Melisa Tripon[1], Timea Kristea[1] and Horia Leonard Banciu[1]

[1]Babeş-Bolyai University, Faculty of Biology and Geology, Department of Molecular Biology and Biotechnology, 5-7 Clinicilor Street, Cluj-Napoca, Romania;
✉Corresponding author, E-mail: cristea.adorjan@gmail.com.

Abstract

Polyhydroxyalkanoates (PHAs) are polyesters produced and stored intracellularly by various microbes during periods with an excess of carbon (C) source and limitation of nitrogen (N) and/or phosphorus (P) nutrients. We aimed to explore the influence of N and P nutrient limitation on the synthesis of PHAs in two *Halomonas* strains. *H. elongata* DSM 2581^T and the environmental strain 2FF used in this study were grown in shake flask cultures (at 37^{o}C and 180 rpm) on liquid mineral DSM 343 medium with 8% (w/v) NaCl and three nutrient limitation conditions: simultaneous N and P limitation, N limitation or P limitation. D-glucose (1% w/v) was used as an excess carbon source in all experiments. Accumulation of PHAs was quantified by the crotonic acid assay. The highest PHA production was evidenced after 72 hours of growth under N limitation (1.56 $g \cdot l^{-1}$) in strain DSM 2581^T and simultaneous N and P limitation (0.7 $g \cdot l^{-1}$) in strain 2FF. Additionally, higher biomass (as cell dry weight) and PHA yields were achieved at 180 rpm as compared to 110 and 140 rpm shaking speeds. This study is a first attempt to investigate the influence of N and P on PHAs synthesis in *Halomonas* spp. cultured at high salinity (8% NaCl), although similar tests were previously conducted on *Halomonas* spp. (Jin *et al.*, 2013; Ilham *et al.*, 2014) but at much lower salinity (0.1% and 1% NaCl, respectively).

Keywords: *Halomonas,* nutrient limitation, polyhydroxyalkanoates, saline.

Acknowledgments: This work was supported by grant from the CNCS–UEFISCDI, project number PN-III-P4-ID-PCE-2016-0303.

References

Ilham, M., Nakanomori, S., Kihara, T., Hokamura, A., Matsusaki, H., Tsuge, T., & Mizuno, K. (2014). Characterization of polyhydroxyalkanoate synthases from *Halomonas* sp. O-1 and *Halomonas elongata* DSM2581: Site-directed mutagenesis and recombinant expression. *Polym Degrad Stabil* **109**: 416-423.

Jin, Y. X., Shi, L. H., & Kawata, Y. (2013). Metabolomics-based component profiling of Halomonas sp. KM-1 during different growth phases in poly (3-hydroxybutyrate) production. *Biores Technol* **140**:73-79.

Isolation and characterization of halophilic mannan-degrading enzyme produced by halophilic archaea

Daichi Hitomi[1,✉], Hiroaki Minegishi[1] and Yasuhiro Shimane[2]

[1]Toyo University Kawagoe Campus, kujirai2100, Kawagoe city Saitama, JAPAN; [2]Japan Agency for Marine-Earth Science and Technology JAMSTEC, Natsushima2-15, Yokosuka city Kanagawa;
*✉**Corresponding author, E-mail: seimeiken.hitomi@gmail.com***

Abstract

Mannans are the major constituents of the hemicellulose fraction in softwoods, being widesread in plant tissues. Mannanase is an important enzyme involved in degradation of mannan, production of bioactive oligosaccharides, and biobleaching of kraft pulp (Moreira & Filho, 2008). Acidic and neutral-pH mannanases are more commonly found, however, few studies of halophilic mannanases are yet known. We aimed at the isolation of halophilic strains capable of producing salt stable mannan-degrading enzymes using commercial salt made in Japan as inoculum. The commercial salt samples were spread on the agar plates and incubated at 37 °C for 3 weeks. In total, six strains were isolated that produced clear zone around colonies on agar plates, containing 0.2% Locust Been Gum (LBG), when flooded with 0.1% Congo-red solution. The result of 16S rRNA gene sequence analysis indicated that the strain ManD738 was similar to *Haloarcula quadrata* strain JCM 11048^T (99.5%); the strains ManD500, ManD722 and ManD381 were similar to *Haloarcula japonica* strain JCM 7785^T (99.2%), and the strains ManD261 and ManD777 were similar to *Halobacterium noricense* strain JCM 15102^T (99.2%). Mannanase activity was determined by measuring the increase of reducing sugar released from mannan using the modified dinitrosalicylic acid (DNS) method. The extracellular mannanase produced from strain ManD738 in supernatant showed optimum NaCl concentration at 3.5 M, optimum temperature at 70 °C, and pH optimum at 7.0. The mannanase produced by strain ManD738 was stable at the temperature of 50-75°C and showed 50% relative activity at 0.5 M NaCl and 4.5 M NaCl, respectively.

Keywords: haloarchaea, halophilic mannanase.

References

Moreira, L. R. S., & Filho, E. X. F. (2008). An overview of mannan structure and mannan-degrading enzyme system. *Appl Microbiol Biotechnol* **79**: 165.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 124)

Production and characterization of halophilic esterase and lipase from halophilic microorganisms

Teppei Ishikawa[1,✉], Hiroaki Minegishi[1,2] and Yasuhiro Shimane[2]

[1]Toyo University Kawagoe Campus, Kuzirai2100, Kawagoe city Saitama, Japan; [2]Japan Agency for Marine-Earth Science and Technology JAMSTEC, Natsushima2-15, Yokosuka city Kanagawa, Japan;
✉Corresponding author, E-mail: s36d01800031@toyo.jp

Abstract

Lipase belongs to the hydrolases and is also known as fat-splitting enzyme, glycerol ester hydrolase or triacylglycerol acylhydrolase. Lipase catalyzes the hydrolysis of triglycerides converting them to glycerol and fatty acids. These are widely used in many industrial fields such as oil and fat processing, fabrication of soap, detergent, chemicals, food, pharmaceuticals, and so on. Halophilic enzymes can be highly active not only under high salinity but also in organic solvents and under high temperature. Therefore, in this study, we aimed to look for novel haloarcheal lipase capable of maintaining enzyme activity even under high salt concentration, at high temperature and in organic solvent enviroments. Ninety-seven strains producing lipase were isolated from the 668 different kinds of commercial salt samples. Results of the 16S rRNA gene sequences analysis of two isolates having high lipase activity especially have indicated that they are closely related to *Pseudomonas halophila* and *Halospina denitrificans*, respectively. They produced clear zones around colonies on agar plates containing 1.0% Tween 80. Optimal activities of crude enzymes from halophilic isolates were at 50-60°C, pH 8.0-9.0, and 20% (w/v) NaCl. Fuetheremore, high activity and stability over broad ranges of temperature (40-70°C), pH (7.0-10.0) and NaCl concentration (15-30%) were observed, showing thermostable and halostable properties of the lipases produced by isolated strains.

Keywords: fatty ester, halophilic lipase, halophilic microorganism, lipid.

Acknowledgements. This work was supported by Toshihiro Nagao of Osaka Municipal Technical Research Institute and Akinobu Etigo.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 125)

Characterization of halophilic cellulase-producing novel haloarchaea

Tsubasa Ito[1,✉], Hiroaki Minegishi[1,2] and Yasuhiro Shimane[2]

[1]Toyo University Kawagoe Campus, Kuzirai2100, Kawagoe city Saitama, Japan; [2]Japan Agency for Marine-Earth Science and Technology JAMSTEC, Natsushima2-15, Yokosuka city Kanagawa, Japan; ✉***Corresponding author, E-mail: s36d01800042@toyo.jp***

Abstract

Cellulose is the largest biological resource on the earth. Cellulose-degrading enzymes are used in many industrial fields such as medical digestive enzymes, bioethanol, and cellulose treatment of wastewater. It is known that halophilic enzymes can be catalytically active in organic solvents and high temperature environments. Haloarchaea are adapted to a life in multiple extreme conditions, and some of them are capable of growth on cellulosic waste as carbon and energy source by producing cellulase. However, halophilic cellulases produced by haloarchaea are rarely reported. Therefore, in this study, we aimed to search for novel haloarchaeal cellulase capable of maintaining enzyme activity even under high salt concentration, high temperature and in organic solvent environments. Sixty-four cellulase-producing strains were isolated from 668 kinds of commercial salt samples. The 16S rRNA gene sequences analysis of strains 434Sel4, 434Sel6 and 434Sel7 were classified as a new genus of haloarchaea. Optimal cellulase activities from these haloarchaeal isolates were found at 60-70°C, pH 7.0-8.0 and 30% NaCl. Furthermore, high activity and stability over broad ranges of temperature (40–70°C), pH (6.0–8.0) and NaCl concentration (15–30%) were observed, showing thermostable and halostable properties of the haloarchaeal cellulases.

Keywords: cellulase, enzymes, haloarchaea.

Acknowledgements. This work was supported by The INOUE ENRYO Memorial Grant, TOYO University.

References

Li, X., & Yu, H. Y. (2013). Characterization of a halostable endoglucanase with organic solvent-tolerant property from *Haloarcula* sp. G10. *Int J Biol Macromol* **62**: 101-106.

Ogan, A., Danis, O., Gozuacik, A., Cakmar, E., & Birbir, M. (2012). Production of cellulase by immobilized whole cells of *Haloarcula*. *Appl Biochem Microbiol* **48**(5): 440-443.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 126)

Isolation and characterization of ultraviolet-resistant haloarchaea

Kazusa Komiyama[1,✉], Hiroaki Minegishi[1] and Yasuhiro Shimane[2]

[1]Toyo University Kawagoe Campus, kuzirai2100, Kawagoe city Saitama, JAPAN; [2]Japan Agency for Marine-Earth Science and Technology JAMSTEC, Natsushima2-15, Yokosuka city Kanagawa;
✉Corresponding author, E-mail: kzs.kk1@gmail.com

Abstract

Halophilic archaea within the class Halobacteria are known to be drought- and ultraviolet irradiation-resistant. Moreover, in recent years numerous haloarchaea have been noted as polyextremophiles. Among these, *Halobacterium salinarum* NRC-1 was reported to be resistant to ultraviolet light and radiation. Ultraviolet light is classified into three types according to their wavelength: the long-wavelength, poorly-penetrating UV-A, the mid-wavelength UV-B that indirectly inflict the damage of intracellular components and the short-wavelength UV-C that directly incurs DNA damage (Jones & Baxter, 2017). Studies on the strain NRC-1 have led to the elucidation of several UV resistance mechanisms, but it was not clear whether many of them are clearly associated with resistance. In this study, we isolated 8 halophilic strains capable of growing under UV light irradiation from 810 different kinds of commercially available salt samples. The 16S rRNA gene sequence analysis revealed that the isolates were closely related to the genus *Halobacterium*, *Halorubrum*, *Haloterrigena*, and *Haloparvum*, respectively. During the log phase cultivation, these strains were UV-irradiated for 60 seconds. Our results suggested that the tested haloarchaeal isolates were equally or even more UV-resistant than the standard *Halobacterium* NRC-1 strain.

Keywords: haloarchaea, ultraviolet light, ultraviolet resistant.

References

Jones, D. L., & Baxter, B. K. (2017). DNA repair and photoprotection: mechanisms of overcoming environmental ultraviolet radiation exposure in halophilic archaea. *Front Microbiol* **8**: 1882.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 127)

The influence of the chloride content on the growth of halophilic bacteria isolated from the saline Lake Letea and their extracellular enzymatic activities

Anca Ioana Lucaci[1,✉], Roxana Cojoc[1], Simona Neagu[1],
Robert Ruginescu[1], Ioan Ardelean[1] and Mădălin Enache[1]

[1]*Institute of Biology Bucharest of the Romanian Academy, 296 Splaiul Independentei, 060031 Bucharest, P.O., Box 56-53, Romania,* ✉***Corresponding author, Email: ioana.lucaci@ibiol.ro***

Abstract

This paper presents preliminary results of an investigation that had as main objective to quantify the seasonal variations of halophilic bacteria in the saline Lake Letea (Romania), which has an alkaline pH and a salt (chloride) concentration that varies in time, and their ability to produce extracellular hydrolytic enzymes. The investigations were performed from October 2016 until May 2018. Numbers of cultivable putative moderately halophilic bacteria were estimated using culture medium MH with 10% NaCl. The recorded number of colony forming units/mL was between 41 (October 2016) and 3.7x103 (April 2017). After a random selection, colonies were grown for three successive passages for purification, and 82 isolates were investigated. Based on their salt requirement and tolerance, 69 isolates were halotolerant and 13 isolates were moderately halophilic bacteria. The generation time was also determined for several isolates. Seventeen percent of the isolates were oxidase-negative and 83% oxidase-positive. Forty eight percent of isolates were catalase-negative and 52% catalase-positive. Presence of extracellular enzymatic activities was tested using MH medium with salinity varying between 0-4 M and supplemented with a substrate to induce the production of the hydrolases tested. The highest hydrolytic activities were recorded for Tween 80, casein and inulin. This study represents a preliminary investigation of the diversity and extracellular enzymatic activities of halophilic bacteria isolated from Lake Letea.

Keywords: halophilic microorganism, hydrolytic enzymes, screening.

Acknowledgements. This work was suported by project no. RO1567-IBB01/2019 from the Institute of Biology Bucharest of the Romanian Academy.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 128)

Isolation and characterization of halophilic cellulase-producing bacteria from soil samples in Japan

Kenta Okunari[1,✉], Hiroaki Minegishi[1] and Yasuhiro Shimane[2]

[1]Toyo University Kawagoe Campus, kuzirai2100, Kawagoe city Saitama, JAPAN; [2]Japan Agency for Marine-Earth Science and Technology JAMSTEC, Natsushima2-15, Yokosuka city Kanagawa;
*✉**Corresponding author, E-mail: okunarikenta@gmail.com***

Abstract
Cellulose is the most naturally abundant biopolymer on the Earth and a low-cost energy source. It consists of a linear homopolysaccharide composed by beta-D-glucopyranose units linked by beta-1,4 glycosidic bonds. Cellulases hydrolyze beta-1,4 linkages in cellulose chains. In nature, complete cellulose hydrolysis is mediated by a combination of three main types of cellulases: endoglucanases, exoglucanases (including cellobiohydrolases), and beta-glucosidase. Cellulases play a major role in industries such as paper, textile, detergent, food, and biorefinery. Halophilic microorganisms are excellent sources for salt stable enzymes that can effectively withstand and react under extreme salinity conditions. For instance, they play an essential role in various fermentation processes that occur in the presence of salt. Recently, we isolated 6 moderate halophilic, CMC-degrading strains from non-saline garden soil samples in Japan. After incubated at 37°C for 3 weeks, they produced clearance zone around colonies on agar plates containing 0.2% carboxymethyl cellulose (CMC), when stained with 0.1 % Congo red on the agar plates. Based on the 16S rRNA gene sequencing, the isolates were found as closely related to *Piscibacillus*, *Halobacillus*, *Thalassobacillus*, *Lentibacillu*s, and *Virgibacillus,* respectively. The activity profile of their enzymes showed the optimum residual activity at temperature 50 °C. The cellulase activity was found to be stabilized in the salt range of 6–15% and thus evident for halophilic nature of the enzyme.

Keywords: cellulase, cellulose, CMCase, enzyme, halophile.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 129)

Halophilic microorganisms producing extracellular hydrolytic enzymes isolated from the Atacama Desert

Robert Ruginescu[1,✉], Cristina Purcărea[1], Roxana Cojoc[1], Simona Neagu[1], Ioana Gomoiu[1], Cristina Dorador[2], Paris Lavin[2] and Mădălin Enache[1]

[1]Institute of Biology Bucharest of the Romanian Academy, 296 Splaiul Independentei, Bucharest, Romania; [2]Laboratorio de Complejidad Microbiana y Ecología Funcional, Instituto Antofagasta, Universidad de Antofagasta, 601 Angamos Av., Antofagasta, Chile;
✉Corresponding author, E-mail: robert.ruginescu@ibiol.ro

Abstract

Since most industrial processes are characterised by harsh physicochemical conditions that rapidly inactivate enzymes from mesophiles, current studies are geared towards the detection of extremophilic microorganisms producing enzymes that exhibit optimal activities in extreme ranges of salt concentration, temperature and pH. Halophilic microorganisms are an important source of salt-tolerant hydrolytic enzymes that can be used in bioremediation processes and in various biotechnological applications. In this context, the aim of our research is to screen the halophilic microorganisms producing hydrolases, isolated from the Atacama Desert, the driest and oldest desert on Earth. In order to isolate halophilic microorganisms, suspensions of salt crystals from the Atacama Desert in dilute salt solutions were inoculated on growth media like MH, JCM168, YGC, MEA and BG11. Screening of isolates for extracellular hydrolases (amylase, protease, cellulase, lipase, inulinase, pectinase) was performed on different assay media supplemented with specific substrates (starch, casein, carboxymethyl cellulose, Tween 80, inulin, pectin). Some isolates were selected with respect to their potential of producing hydrolases and studied in detail. The results of the present study represent a preliminary, but essential, step to identify novel biological sources of salt-tolerant enzymes, which would be of great importance particularly in biotechnology. Further investigations should be directed towards the biochemical characterization of these extremozymes and the cloning of encoding genes in order to elucidate the salt tolerance mechanisms and to obtain the best hydrolases producers, respectively.

Keywords: Atacama Desert, halophiles, hydrolytic enzymes, salt tolerance.

Acknowledgements. This research was financially supported by project no. RO1567-IBB05/2018 from the Institute of Biology Bucharest of the Romanian Academy.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 130)

Purification and structural determination of anti-cancer molecule extracted from an extremely halophilic bacterium, *Salinivenus iranica*

Seyed Abolhassan Shahzadeh Fazeli[1, 2, ✉] and Atefeh Safarpour[3]

[1]Microorganisms Bank, Iranian Biological Resource Centre (IBRC), ACECR, Tehran, Iran; [2]Department of Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, ACECR, Tehran, Iran; [3]Extremophiles Laboratory, Department of Microbiology, School of Biology, College of Science, University of Tehran, Tehran, Iran; ✉***Corresponding author, E-mail: shfazeli@yahoo.com***

Abstract

In a drug discovery process from halophiles, *Salinivenus iranica* crude metabolites showed a great cytotoxic activity against breast cancer cell lines including MCF-7, MDA-MB-468, and MDA-MB-231. HPLC analysis and downstream MTT assays were done to purify the active component of the *S. iranica* metabolites. Moreover, FT-IR and LC-MS analysis were done to structural detection of the effector molecule of the selected metabolite. To find the effective molecule in *S. iranica* supernatant metabolite, the metabolite was fractionated into 31 fractions by four solvents: hexane, ethyl acetate, water, and butanol. The viability of human breast cancer cells was assayed in the presence of each fraction with the concentration of 100 µg/mL. The results showed that fraction number 6 of hexane had reduced the viability of all cell lines to 1% while the other 30 fractions had no significant effect on cell lines viability. The mass spectrophotometry determined that molecular weight (MW) of this fraction was about 1961.78 Da. In FT-IR analysis, three types of functional molecules were found in hexane fraction number 6. In 2950 – 2850 cm^{-1}, Alkyl C-H Stretch was detected which are usually less useful in determining structure because of fairly ubiquitination. In 3500 - 3300 cm^{-1}, Amine N-H Stretch was detected which is a primary amine, as primary amines produce two N-H stretch absorptions, secondary amides only one, and tertiary none. In 3550 – 3200 cm^{-1}, Alcohol/Phenol O-H Stretch was detected. In conclusion, based on its unique features, the active competent of *S. iranica* metabolite could be a novel component against breast cancer and additional studies and full characterization of this effector molecule could result in a new drug with strong anti-cancer stem cell effects on breast cancer.

Keywords: breast cancer, drug discovery, LC-MS, *Salinivenus iranica*.

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 131)

Casting light on polysaccharolytic potential of haloarchaea

Margarita Shevchenko[1,✉], Valery Lysun[1], Stanislav Sukhikh[1],
Enzo Messina[2] and Michail Yakimov[2]

[1]*IKBFU, Kaliningrad, Russia;* [2]*Istituto per l'Ambiente Marino Costiero, CNR, Messina, Italy;*
✉***Corresponding author, E-mail: lionsorciere@gmai.com***

Abstract

The solar salterns located close to the island of Mozia (Sicily) represent a potential source of halophilic glycoside hydrolases capable of degrading insoluble natural polysaccharides such as chitin, cellulose and xylan. This research aimed at searching for new hydrolytic enzymes. In 2017, we collected endoevaporitic sediments from various evaporation ponds with a salinity 170-270 PSU. Halophilic archaea and bacteria from the samples were enriched and cultivated on mineral medium (240 PSU) with the addition of chitin, as the sole carbon source. To get insights into metabolic capacities of the enrichment with the greatest hydrolytic activity, we performed shotgun metagenome analysis. The analysis for the searching of genes encoding chitinase-related domains was done using CAZy database, HMMER and dbCAN. As a result, the archaeal genome of *Halomicrobium* sp. LC1Hm was assembled. *Halomicrobium* sp. LC1Hm contains high number of genes apparently involved in polysaccharide metabolism, including genes coding for 26 glycosyl hydrolases (GHs), seven of which belong to class III of GH18 family (EC 3.2.1.14). All of inferred GHs possess the N-terminal Tat-secretion signals and the ChtBD3 chitin-binding domains. This type of endochitinases breaks down the chitin microfibrils at internal sites forming low molecular weight chitodextrins. These are the substrates for a putative extracellular GH20 family protein Hmb_0796, annotated as β-N-acetylglucosaminidase (EC 3.2.1.52). We foresee strong biotechnological importance of the project's outcomes due to developing novel methods for processing insoluble natural polymers.

Keywords: halophiles, halophilic glycoside hydrolases, metagenomic sequencing.

Acknowledgements. his work was suported by grant of RFBR № 18-34-00802. Travel was fundedby IKBFU «5 top 100»

STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, 64, 1, 2019 (p. 132)

Exploring and characterization of extracellular halophilic amylase produced by haloarchaea

Shuuhei Umezawa[1,✉], Hiroaki Minegishi[1] and Yasuhiro Shimane[2]

[1]Toyo University Kawagoe Campus, kuzirai2100, Kawagoe city Saitama, Japan; [2]Japan Agency for Marine-Earth Science and Technology JAMSTEC, Natsushima2-15, Yokosuka city Kanagawa;
✉Corresponding author, E-mail: s36d01800064@toyo.jp

Abstract

The enzymes produced by halophilic archaea are capable of withstanding extreme conditions such as high temperature, high or low pH, organic solvent and high salt concentration environments. Starch degrading enzyme, amylase, catalyzes the hydrolysis of internal alpha-1,4 glycosidic bonds in polysaccharides with the retention of alpha anomeric configuration in the products. Amylases are widely used during processing of food, medicines, paper, textile, etc. Amylases are increasingly studied due to the relative ease of large-scale production. Halophilic archaea within the class Halobacteria include a large group of aerobic microbes that live and grow in hypersaline environments. Many halophilic archaea are known to produce amylase. Research on chracterization of halophilic amylase, however, is hardly ever reported (Santorelli *et al*., 2016). We explored halophilic archaea producing amylase with basic halophilic archaea medium that contained soluble starch. We isolated an extremely halophilic, starch-degraging strain U243S from sea salt samples made in Okinawa, Japan. The extracellular amylase in supernatant had pH optimum at 7.0, optimum temperature at 60° C, optimum NaCl concentration at 20% (w/v), and showed 80% relative activity after 2 hours of heating at 50° C. The 16S rRNA partial gene sequence of strain U243S (1353 bp) indicated its relatedness to *Halomicroarcula pellucida* strain BNERC31^T (100% similarity) (Echigo *et al*., 2013).

Keywords: amylase, haloarchaea, halophilic amylase.

References

Santorelli, M., Maurelli, L., Pocsfalvi, G., Fiume, I., Squillaci, G., La Cara, F., del Monaco, G., & Morana, A. (2016). Isolation and characterisation of a novel alpha-amylase from the extreme haloarchaeon *Haloterrigena turkmenica*. *Int J Biol Macromol* **92**: 174-184.

Echigo, A., Minegishi, H., Shimane, Y., Kamekura, M., Itoh, T., & Usami, R. (2013). *Halomicroarcula pellucida* gen. nov., sp. nov., a non-pigmented, transparent-colony-forming, halophilic archaeon isolated from solar salt. *Int J Syst Evol Microbiol* **63**(10): 3556-3562.

Authors Index