R2K Data Compliance Plan
Costantino Vetriani, Richard Lutz
OCE-0327353
Collaborative research: Integrated studies of biological community structure at deep-sea hydrothermal vents

Specific analyses on datasets

In 2003, we (R. Lutz, G. Luther, C. Vetriani, and T. Shank) proposed an integrated research program to identify first order biological-geochemical interactions in the context of previously observed patterns of faunal succession at deep-sea hydrothermal vents through time-series studies at the East Pacific Rise near 9° 50'N that combined molecular genetic characterization of microbial communities and metazoan colonists with in-situ measurements of H2, H2S, O2, H2O2, pH and temperature. Initial results from the microbial component of this integrative project have been published (Vetriani et al., 2004, Vetriani et al., 2005, Hugler et al., 2007, Voordeckers et al., 2008, Lutz et al., 2008, Crespo-Medina et al., 2009, Crespo-Medina et al., in press). Following the 2005-06 eruptive event on the EPR, we redesigned our experimental strategy by deploying artificial colonization substrates to monitor the colonization process. Chemical data were collected along with microbial and faunal samples and are currently being processed. The final integrative analyses are the subject of a currently pending proposal (submitted to R2K in April 2009).

Our PI group has submitted Level 1 and Level 2 metadata to the Ridge 2000 Data Management Office (DMO), and derived dataset relative to the microbial component of this project that will be linked to the DMO are listed in the Table at the end of this document. Sites where specific microorganisms were isolated will be shown on the Marine Geoscience Data System (MGDS).

References Relevant to the PIs work above

Crespo-Medina, M., A. D. Chatziefthimiou, R. Cruz-Matos, I. Perez-Rodriguez, T. Barkay, R. A. Lutz, S. V., and C. Vetriani. *Salinisphaera hydrothermalis* sp. nov., a novel mesophilic, facultative chemotrophic *Gammaproteobacterium* from deep-sea hydrothermal vents. *Intl. J. Syst. Evol. Microbiol*., in press.

Voordeckers, J., Crespo-Medina, M., and Vetriani, C. Detection of the periplasmic nitrate reductase (NapA) in thermophilic, chemolithoautotrophic epsilon-proteobacteria and in deep-sea hydrothermal vent microbial communities. Submitted to *Appl. Env. Microbiol.*

Crespo-Medina, M., Chatziefthimiou, A.D., Bloom, N.S., Luther, G.W, Wright, D.D., Reinfelder, J.R., Vetriani, C., and Barkay, (2009). T. Adaptation of Chemosynthetic Bacteria to Elevated Mercury Concentrations in Deep-Sea Hydrothermal Vents. *Limnol. Ocean*. 54:41-49.

Lutz, R.A., Shank, T.M., Luther, G.W., Vetriani, C., Tolstoy, M., Nuzzio, D.B., Moore, T.S., Waldauser, F., Crespo-Medina, M., Chatziefthimiou, A., Annis, E.R., and Reed, A.J. (2008). Interrelationships between vent fluid chemistry, temperature, seismic activity and biological community structure at a deep-sea hydrothermal vent along the East Pacific Rise. *Journal of Shellfish Research* 27:177-190.

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Nees, H.A., Moore, T.S., Mullaugh, K.M., Holyoke, R.R., Janzen, C.P., Ma, S., Metzger, E., Waite, T.J., Yücel, M., Lutz. R.A., Shank, T.M., Vetriani, C., Nuzzio, D.B., and Luther, G.W. (2008). Hydrothermal vent mussel habitat chemistry, pre- and post-eruption at 9°N50' North on the East Pacific Rise. *Journal of Shellfish Research* 27:169-175.

Hügler, M., Huber, H., Molyneaux, S.J., Vetriani, C., and Sievert, S.M. (2007). Autotrophic CO_2 fixation via the reductive tricarboxylic acid cycle in different lineages within the phylum *Aquificae*: Evidence for two ways of citrate cleavage. *Environmental Microbiology* 9:81-92.

Van Dover, C. L., and R. A. Lutz. 2004. Experimental ecology at deep-sea hydrothermal vents: A perspective. Journal of Experimental Marine Biology and Ecology, 300:273-307.

Vetriani, C. Chew, Y.S., Miller, S.M., Yagi, J., Coombs, J., Lutz, R.A., and Barkay, T. (2005). Mercury adaptation among bacteria from a deep-sea hydrothermal vent. *Appl. Environ. Microbiol.* 71:220-226.

Vetriani, C., Speck, M.D., Ellor, S.V., Lutz, R.A., and Starovoytov,V. (2004) *Thermovibrio ammonificans* sp. nov., a thermophilic, chemolithotrophic, nitrate ammonifying bacterium from deep-sea hydrothermal vents. *Intl. J. Syst. Evol. Microbiol*. 54:175-181.

Anticipated products

Voordeckers, J.W., Crespo-Medina, M., Lutz, R.A. and Vetriani, C. Detection and phylogenetic analysis of the periplasmic nitrate reductase (NapA) in chemolithoautotrophic *Epsilonproteobacteria* and microbial communities from deep-sea hydrothermal vents. Revised manuscript submitted to *Appl. Environ. Microbiol.*

Shank, T.M., Govenar, B., Von Damm, K.L., Fornari, D.J., Lilley, M., Luther, G.W., Lutz, R.A., Tolstoy, M. and C. Vetriani. Temporal and spatial dynamics of faunal community structure in relation to fluid geochemistry, microbial community structure and disturbance on the East Pacific Rise (in preparation for *Geochemistry Geophysics Geosystems*, special theme: "Recent Volcanic Eruptions, Properties, and Behavior of the Fast-Spreading East Pacific Rise at 8º-11ºN").

Additional Results Associated with this Program

Andrianasolo, E., Haramaty, L., Rosario-Passapera, R., Bidle, K., White, E., Vetriani, C., Falkowski, P., Lutz, R. Ammonificin A and B, hydroxyethylamine chroman derivatives with antimicrobial and apoptosis-induction activities from a cultured marine hydrothermal vent bacterium, *Thermovibrio ammonificans*. Submitted to *J. Nat. Prod.*

Reed, A. J., Dorn, R., Van Dover, C. L., Lutz, R. A. and Vetriani, C. Phylogenetic diversity of methanogenic, sulfate-reducing and methanotrophic prokaryotes from deep-sea hydrothermal vents and cold seeps. *Deep-Sea Res*, in press.

Rona, P. A., Seilacher, A., de Vargas, C., Gooday, A. J., Bernhard, J. M., Bowser, S., Vetriani, C., Wirsen, C. O., Mullineaux, L., Sherrel, R., Grassle, J. F., Low, S., and Lutz, R. A. *Paleodictyon nodosum*, a living fossil on the deep sea floor. *Deep-Sea Res*, in press.

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Lutz, R.A., A.G. Collins, E.R. Annis, A.J. Reed, K. Bennett, K.M. Halanych, R.C. Vrijenhoek, (2006). Stauromedusan populations inhabiting deep-sea hydrothermal vents along the southern East

Pacific Rise. Cah. Biol. Mar., 47:409-413.

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Lutz, R.A., and D. Katharine Coykendall. (2008). (Guest Editors) Life in Extreme Environments: A Tribute to Melbourne Carriker, Gentleman Malacologist. Journal of Shellfish Research, Vol. 27, 246.

Chatziefthimiou, A.D., Crespo-Medina, M., Wang, Y., Vetriani, C., and Barkay, T. (2007). The isolation and initial characterization of mercury resistant chemolithotrophic and thermophilic bacteria from mercury rich geothermal springs. *Extremophiles* 11:469-479.

Voordeckers, J.W., Starovoytov, V., and Vetriani, C**.** (2005). *Caminibacter mediatlanticus* sp. nov., a thermophilic, chemolithoautotrophic, nitrate ammonifying bacterium isolated from a deep-sea hydrothermal vent on the Mid-Atlantic Ridge. *Intl. J. Syst. Evol. Microbiol*. 55:773-779.

Data Set	Metadata	Specific Analyses	Storage	Status/ Delivery Date	References
Microbial culture collection including > 400 pure cultures from deep-sea environments (>200 from the EPR)	From Alvin dives during 4 cruises: 2004 (AT11-10), 2005 (AT11-26), 2006 (AT15-06), 2007 (AT15-15)	Isolation of microorganisms	Maintained as frozen stocks in the Deep-Sea Microbiology Lab	List of microorganisms in the culture collection ready to be deposited in DMO (May 2009). Active cultures available upon request to Costa Vetriani.	Vetriani et al., 2004; Crespo-Medina et al., in press; Perez-Rodriguez et al, submitted
Genome sequences from two vent organisms	From AT05-03, AT03-50	DNA sequencing with funding from the G. & B. Moore Foundation and the Department of Energy	One genome sequence publicly available from GenBank (DSM 16658, accession # ABCJ00000000) One genome currently scheduled for sequencing (DSM 15068)	One genome sequence available via link to GenBank. One genome will be made available when ready (expected by early 2010)	
Pure cultures	From AT05-03, AT03-50, AT11-10	Characterization and description of new microbial species	Deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH and in the Japanese Culture Collection. Available publicly	Available via link to the DSMZ and JCM (DSM 15068, JCM 12110; DSM 16658, JCM 12641; DSM 21483, JCM 15514 (available July 2009)	Vetriani et al., 2004; Voordeckers et al., 2005; Crespo-Medina et al., in press
Microbial colonization experiments	From Alvin dives during 4 cruises: 2004 (AT11-10), 2005 (AT11-26), 2006 (AT15-06), 2007 (AT15-15)	Taxonomic and molecular identification	Frozen, preserved in 95% ethanol	10/2011 for final integrated data	
Gene sequences and nucleic acids from microorganisms	From Alvin dives during 4 cruises: 2004 (AT11-10), 2005 (AT11-26), 2006 (AT15-06), 2007 (AT15-15)	Phylogenetic analyses	Sequences in GenBank; nucleic acids frozen	Publicly available via link to GenBank; Nucleic acids available available upon request to Costa Vetriani.	
Mussel removal/ caged colonization substrates-macrofaunal colonists	Three deployments: 2004 (A3998, A4001, A4010)	Direct counts and molecular identification of colonists	Frozen, preserved in 95% ethanol	Final species lists available now on the R2K Data Portal	Lutz et al. 2008

Sample requests

Samples can be obtained by contacting Costantino Vetriani (732-932-6555 x373, vetriani@marine.rutgers.edu) or Richard Lutz (732-932-6555 x200, rlutz@marine.rutgers.edu).

Links: GenBank: http://www.ncbi.nlm.nih.gov/Genbank/; DSMZ: http://www2.dsmz.de/index.htm; JCM: http://www.jcm.riken.go.jp/; Deep-Sea Microbiology Lab