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IN REPLY REFER TO:

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N00014-89-J-1048
27 February, 1992

From: Office of Naval Research Resident Representative, Seattle
To: ONR Scientific Officer, Dr. Randall S. Alberte, Code 1123B, Oceanic
Biology, Office of the Chief of Naval Research, Ballston Tower
1, 800 North Quincy Street, Arlington, VA 22217-5000

Subj: REQUEST FOR FINAL TECHNICAL APPROVAL, GRANT N00014-89-J-1048, R&T
PROJECT CODE: 423a001---02; THE UNIVERSITY OF WASHINGTON; PRINCIPAL
INVESTIGATOR IS DR. JODY W. DEMING, SCHOOL OF OCEANOGRAPHY

1. This office is in the process of closing subject contract. We have been advised that the final technical report has been submitted.
2. So that closeout may continue, please provide this office with information as to whether technical requirements have been performed satisfactorily.

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Eleanor A. Dixon
ELEANOR A. DIXON
Procurement Assistant

cc:
DTIC (w/copy of final technical report)
***** DO NOT DETACH *****
FIRST ENDORSEMENT on ONR ltr., 27 February, 1992

From: ONR Scientific Officer, Dr. Randall S. Alberte, Code 1123B
To: Office of Naval Research, Seattle

1. Returned for necessary action.
2. I certify that all technical requirements under subject contract are:

____ Satisfactory
____ Unsatisfactory
____ Comments:

92-05937



02 3 05 006

Date

Scientific Officer

UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON 98195

School of Oceanography, WB-10

Date: February 20, 1992

To: Randall Alberte, Scientific Officer, ONR; and
Defense Technical Information Center

From: Jody W. Deming, Associate Professor 
School of Oceanography, WB-10, University of WA

Subj: Final Technical Report
ONR Grant N00014-~~88~~-J-1048 (UW Budget 62-4981)
"Bacterial Responses to Extreme Temperatures and
Pressures and to Heavy Organic Loading"

OBJECTIVES OF THE GRANT

The goals of this project centered around two areas of research: 1) the study of hyperthermophilic archaeobacteria from submarine hydrothermal vents on the East Pacific Rise; and 2) the study of physiologically diverse microbial populations associated with a whale carcass partially buried in sediments of the Santa Catalina Basin. Specific objectives of the first area of research included the following:

- (1) Completion of analyses of all geochemical, biochemical and microbiological measurements made on smoker fluid samples obtained during ALVIN dives to the Endeavour Segment of the Juan de Fuca Ridge (under earlier funding) in order to assess the potential existence of a subcrustal biosphere;
- (2) Further characterization of DNA from some of those smoker samples and selected isolates to assess phylogenetic origin;
- (3) Determination of the upper temperature and pressure limits for growth and survival of hyperthermophilic archaeobacteria obtained during earlier cruises; and
- (4) Tests for the production of thermostable extra-cellular enzymes during growth and survival studies at extreme conditions:

The specific objective of the second area of research included tests of two hypotheses:

- 1) Physiological types of bacterial-invertebrate symbioses in the deep sea directly reflect available energy sources; and

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- 2) Deep-sea conditions of low temperature and elevated pressure do not limit bacterial responses to intense organic loading.

ACCOMPLISHMENTS:

With regards to research on hyperthermophilic vent organisms, we successfully completed analyses of the DNA and corresponding geochemical data obtained from smoker samples during the 1988 ALVIN dive program on the Endeavor Segment. These analyses resulted in: 1) the first statistically significant evidence for a vast subsurface biosphere at deep-sea spreading zones; 2) an important section in an invited book chapter (Baross and Deming, 1992; see below) that reviews all available information on thermophilic bacteria from hydrothermal environments; 3) an invited presentation at the 1991 GSA meetings in San Diego that is now the basis for a manuscript in preparation; 4) an invitation to join the French microbiology expedition MICROSMOKE, planned for Mid-Atlantic vents in 1994, to again deploy our in situ Ti incubator at active smokers on the seafloor in search of microbial activity at superheated temperatures.

The temperature and pressure growth characteristics of two strains of hyperthermophilic archeobacteria were determined and published, along with the prediction (based on those characteristics) that microbial habitats exist deep below seafloor spreading zones. Another manuscript, identifying the organisms as members of the genus *Thermococcus* on the basis of 16S rRNA sequences, is in draft form.

The study the thermostability of proteins produced by hyperthermophilic bacteria in culture evolved into more fundamental studies of protein formation and stability under extreme conditions. In collaboration with geochemist M. Engel at the University of Oklahoma, organic chemist S. Macko at the University of Virginia and geophysicist E. Schock at Washington University in St. Louis, we conducted a series of high temperature/pressure experiments in my laboratory on the stability and racemization of amino acids and peptide bonds under simulated hydrothermal conditions, including the presence of solid minerals. This work has resulted in: 1) evidence that peptides are more stable under extreme conditions than isolated amino acids, in support of J. Baross' (University of Washington) theory on the origin of life and in keeping with H. Helgeson's (Berkeley) theory on metastable conditions in the subsurface regime; 2) a joint presentation at the 1991 GSA meetings in San Diego; 3) a joint manuscript in preparation; and 4) plans to submit a joint proposal to explore further both the experimental and theoretical possibilities.

In pursuit of molecular studies of vent (and other) bacteria, a masters-level molecular biologist (S. Carpenter) was

hired and trained in the art of culturing "extremophiles" from the deep sea. She, in turn, established connections to the molecular biology facility in the Department of Botany, where the equipment we lack is available, and completed the extraction and purification of DNA from all of our hyperthermophilic cultures, as well as from previously collected smoker fluids, in preparation for more sophisticated work to identify their phylogenetic and some physiological characteristics. The pay-off from this ambitious goal lies well beyond the timing and funding constraints of this ONR grant; however, we have made a notable beginning.

With regards to research on the impact of heavy organic loading at the seafloor, my laboratory group joined the C. Smith (University of Hawaii) NSF ALVIN project at the dead whale site in the Santa Catalina Basin in Feb 1991. Our efforts during and after this cruise resulted in: 1) the documented persistence of a chemoautotrophic basis for invertebrate communities on the whale bones; 2) experiments to confirm the simultaneous presence (and novelty) of endosymbiotic nitrifiers and sulfur-oxidizers in animal tissues (analyses ongoing); 3) manuscripts in preparation on the effects of organic enrichment on microbial parameters in these deep-sea sediments; and 4) a unique educational opportunity for my graduate students to adapt intertidal microbiological techniques to research on deep-sea sediments (methods manuscript in preparation).

PUBLICATIONS RESULTING FROM THE GRANT:

- Smith, C.R., H. Kukert, R.A. Wheatcroft, P.A. Jumars, and J.W. Deming. 1989. Vent fauna on whale remains. *Nature* 341: 27-28.
- Straube, W.L., J.W. Deming, C.C. Somerville, R.R. Colwell, and J.A. Baross. 1990. Particulate DNA in smoker fluids: Evidence for existence of bacterial populations in hot hydrothermal systems. *Appl. Environ. Microbiol.* 56: 1440-1447.
- Jumars, P.J., L. Mayer, J.W. Deming, and J.A. Baross. Deep-sea deposit-feeding strategies suggested by environmental and feeding constraints. 1990. *Phil. Trans. R. Soc. Lond. A* 331: 85-101.
- Rowe, G.T., M. Sibuet, J.W. Deming, and A. Khripounoff. 1990. Organic carbon turnover time in deep-sea benthos. *Prog. Oceanogr.* 24: 141-160.
- Reysenbach, A.-L., and J.W. Deming. 1991. Effects of hydrostatic pressure on growth of hyperthermophilic archaeobacteria from the Juan de Fuca Ridge. *Appl. Environ. Microbiol.* 57: 1271-1274.
- Allison, P.A., C.R. Smith, H. Kukert, J.W. Deming, and B.A. Bennett. 1991. Deep-water taphonomy of vertebrate carcasses: a whale skeleton in the bathyal Santa Catalina Basin. *Paleobiol.* 17: 78-89.

- Deming, J.W., and P.L. Yager.** 1992. Natural bacterial assemblages in deep-sea sediments: Towards a global view. In: G.T. Rowe and V. Pariente, eds., *Deep-Sea Food Chains and the Global Carbon Cycle*, NATO Conference Proceedings, Kluwer Academic Publishers, pp. 11-27.
- Rowe, G.T., M. Sibuet, J.W. Deming, A. Khripounoff, J.H. Tietjen, S.A. Macko, and R. Theroux.** 1992. "Total" Sediment Biomass and Preliminary Estimates of Organic Carbon Residence Time in Deep-Sea Benthos. *Mar. Ecol. Prog. Ser.* (in press).
- Deming, J.W., and J.A. Baross.** 1992. The early diagenesis of organic matter: Bacterial activity. In: M.H. Engel and S.A. Macko, eds., *Organic Geochemistry*, Vol. 6, Topics in Geobiology, Plenum Press, New York (in press).
- Baross, J.A., and J.W. Deming.** 1992. Bacterial growth at high temperatures: Isolation and taxonomy, physiology, and ecology. In D.M. Karl, ed., *Microbiology of Deep-Sea Hydrothermal Vent Habitats*, Telford Press, Caldwell, New Jersey (invited book chapter, in press).

MANUSCRIPTS IN PREPARATION:

- Reysenbach, A.-L., J.W. Deming, D.B. Hedrick, D.C. White, and N.R. Pace.** 1992. Characterization of deep-sea hyperthermophilic members of the genus *Thermococcus* from the Juan de Fuca Ridge. (In draft form for *Syst. Appl. Microbiol.*)
- Floury, L., J. Deming, A. Deuff, T. Le Moign, and D. Prieur.** 1992. A new pressurized water sampler for the study of microbial activity in hot, deep-sea hydrothermal vents. (In draft form for *Deep-Sea Res.*)
- Deming, J.W.** 1992. Deep-sea and laboratory evidence for the existence of microorganisms at high temperatures and pressures (in preparation for Special Issue of *Cosmochem. Geochem. Acta*, based on 1991 invited GSA abstract).
- Engel, M.H., Y. Qian, J.W. Deming, S. Carpenter, and S.A. Macko.** 1992. Diagenesis of amino acids and peptides at elevated temperatures and pressures (in preparation for Special Issue of *Cosmochem. Geochem. Acta*, based on 1991 invited GSA abstract).
- Deming, J.W., A.-L. Reysenbach, D.J. Niemer, S.A. Macko, and C.R. Smith.** 1992. Microbial associations with megafauna colonizing a whale carcass on the seafloor: Specific analogs to hydrothermal vent environments (in preparation, based on 1989 ASM abstract).
- Deming, J.W., P.L. Yager, S. Carpenter, and J. Gaylord.** 1992. Bacterial density profiles in sediments impacted by a whale carcass. (in preparation, based on 1989 ASM abstract).
- Ritzrau, W., P.L. Yager, and J.W. Deming.** 1992. Substrate hydrolysis and uptake rates by microbial populations in organic-rich sediments of the Santa Catalina Basin (in preparation).

ABSTRACTS:

- Reysenbach, A.-L., and J.W. Deming. 1989. Extremely thermophilic bacteria from submarine hydrothermal vents on the Juan de Fuca Ridge. Annual Meeting, American Society for Microbiology, New Orleans.
- Deming, J.W., A.-L. Reysenbach, D. Niemer, H. Kukert, and C.R. Smith. 1989. Microbial ecology of the first whale carcass ever discovered on the deep-sea floor. Annual Meeting, American Society for Microbiology, New Orleans.
- Deming, J.W., A.-L. Reysenbach, and J.A. Baross. 1989. Biotechnological Potential of Bacteria from Deep-Sea Environments. Annual Meeting, Society for Industrial Microbiology, Seattle.
- Reysenbach, A.-L., and J.W. Deming. 1989. Survival of deep-sea thermophiles under extreme temperatures and vent pressure. International Symp. on Microb. Ecol., Kyoto, Japan.
- Deming, J.W., J.A. Baross, W.L. Straube, and R.R. Colwell. 1989. Evidence for intact microorganisms in superheated smoker fluids: Particulate DNA and preliminary results from a new in situ incubator. Fall Meeting, AGU, San Francisco.
- Deming, J.W., A.-L. Reysenbach, D.J. Niemer, S.A. Macko, and C.R. Smith. 1990. Microbial associations with megafauna colonizing a whale carcass on the seafloor: Specific analogs to hydrothermal vent environments. Ocean Sciences Meeting, American Society of Limnology and Oceanography, New Orleans.
- Deming, J.W. 1991. Natural bacterial assemblages in deep-sea sediments: Towards a global view. NATO Conference on Deep-Sea Food Chains and the Global Carbon Cycle, College Station, Texas
- Deming, J.W. 1991. Deep-sea and laboratory evidence for the existence of microorganisms at high temperatures and pressures. Invited speaker, Organic Matter Survivability at High Temperatures, Geological Society of America, San Diego.
- Engel, M.H., Y. Qian, S.A. Macko, and J.W. Deming. 1991. Diagenesis of amino acids and peptides at elevated temperatures and pressures. Organic Matter Survivability at High Temperatures, Geological Society of America, San Diego.

Statement A per telecom
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Accession For	
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