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OMB No. 0704-0188

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1. A

16

3. REPORT TYPE AND DATES COVERED

4. TITLE AND SUBTITLE

Regulation of Light Output and Identification of Luminous Bacteria from free-living and particle-associated bacterial Assemblages

5. FUNDING NUMBERS

N00014-91-J-1885
R&T Code = 441p006

6. AUTHOR(S)

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8. PERFORMING ORGANIZATION REPORT NUMBER

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Office of Naval Research
800 N. Quincy St.
Arlington, VA 22217-5000

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

NTIS CRA&I
DTIC TAB
Unannounced
Justification

4
☐

11. SUPPLEMENTARY NOTES

DTIC
NOTE
DEC 23 1993

By
Distribution /

12a. DISTRIBUTION / AVAILABILITY STATEMENT

Distribution Unlimited

12b. DISTRIBUTION CODES

Dist Avail and/or Special

A-1

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COPY
INSPECTED

13. ABSTRACT (Maximum 200 words)

Samples from a culture of the marine nitrogen-fixing bacteria Vibrio natriegens were collected from aerobic/combined-nitrogen replete conditions and at regular intervals after shifting the culture to anaerobic/combined-nitrogen absent conditions. RNA and proteins were extracted from the samples for filter-binding assays using a 1.9 kb nifH probe and antisera to nitrogenase. Results indicated that nifH mRNA and nitrogenase began to appear 100-120 min after shifting the culture from aerobic/combined-nitrogen replete to anaerobic/combined-nitrogen absent conditions. After 6-9 h (early log growth) nifH mRNA disappeared, whereas nitrogenase remained throughout log growth up to 18 h after shift. Additionally, both nifH mRNA and nitrogenase disappeared within 40 min after shifting the culture from nitrogen-fixing to non-nitrogen-fixing conditions; nifH mRNA disappeared within 10 min after the shift in a subsequent experiment

93 12 21 1 33

93-30793



14. SUBJECT TERMS

Nitrogenase, nitrogen-fixing, Vibrio natriegens, marine bacteria

NUMBER OF PAGES

3

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT

Unclassified

18. SECURITY CLASSIFICATION OF THIS PAGE

Unclassified

19. SECURITY CLASSIFICATION OF ABSTRACT

Unclassified

20. LIMITATION OF ABSTRACT

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ANNUAL REPORT

Grant # N00014-91-J-1885

R&T Code = 441p006

PRINCIPAL INVESTIGATOR: Dr. James A. Coyer

INSTITUTION: University of California-Los Angeles

GRANT TITLE: Regulation of Light Output and Identification of Luminous Bacteria From Free-living and particle-associated Bacterial Assemblages

REPORTING PERIOD: 1 January 1993 - 31 December 1993

AWARD PERIOD: 1 January 1993 - 31 December 1993

OBJECTIVE: To determine how changes in environmental conditions affect gene transcription and translation.

APPROACH: Samples from a culture of the marine nitrogen-fixing bacteria *Vibrio natriegens* were collected from aerobic/combined-nitrogen present conditions and at regular intervals (40 min-15 hrs) after shifting the culture to anaerobic/combined-nitrogen absent conditions. RNA and proteins were extracted from the samples for filter-binding assays (northerns, westerns) using a 1.9 kb *nifHD* probe and antisera to nitrogenase. In situ hybridization techniques are used to visualize those cells from a population of cells that are potential nitrogen-fixers or are actively fixing nitrogen. Primers were designed to amplify (via the polymerase chain reaction) a 296 bp *nifH* segment from the 1.9 kb *nifHD* insert excised from the plasmid pSA30 using restriction enzymes *EcoRI* and *BamHI*.

ACCOMPLISHMENTS (last six months): Results of the shift experiments indicate that *nifH* mRNA and nitrogenase begin to appear 100-120 minutes after shifting the culture from aerobic/combined-nitrogen present to anaerobic/combined-nitrogen absent conditions. After 6-9 hrs (early log growth), *nifH* mRNA disappeared, whereas nitrogenase remained throughout log growth up to 18 hrs after shift. Additionally, both *nifH* mRNA and nitrogenase disappeared within 40 minutes after shifting the culture from anaerobic (nitrogen-fixing) to aerobic (non-nitrogen-fixing) conditions; *nifH* mRNA disappeared within 10 minutes after the shift in a subsequent experiment. The hybridization pattern to RNA from nitrogen-fixing bacteria using the PCR-derived 296 bp probe to a conserved portion of *nifH* is identical to the hybridization pattern revealed with a 1.9 kb probe complementary to *nifH* and partial *nifD*.

SIGNIFICANCE: Marine nitrogen-fixing bacteria respond rapidly to anaerobic, low combined nitrogen conditions by simultaneously transcribing and translating *nif* genes to nitrogenase. However, the mRNA is soon degraded, whereas the nitrogenase remains active for many hours. In situ hybridization techniques, therefore, must utilize both nucleic acid probes as well as antisera to nitrogenase when resolving nitrogen-fixing species from a mixed population of bacteria.

WORK PLAN (next 6 months): The specific objective of the next work period is to refine the 296 bp probe for in situ hybridizations. One approach is to label the 296 bp probe with digoxigenin (DIG) during PCR amplification. After in situ hybridization to *nifH*, the DIG-labeled probe can be detected with fluorescently-labeled anti-DIG using epifluorescence microscopy. Another approach is to use the PCR primers, *nifH* mRNA from a bacterial culture harvested during nitrogen-fixation, and reverse transcriptase to produce a cDNA probe complementary to the 296 bp conserved region of *nifH*. A cDNA probe will increase the efficiency of hybridization and signal strength.

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nifH mRNA

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Nitrogenase

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OBJECTIVES

- Determine time course of nifH mRNA after shift to Nitrogen-fixing conditions
- Determine time course of nitrogenase after shift to Nitrogen-fixing conditions
- Identify Nitrogen-fixing bacteria with DNA probe to nif gene

ACCOMPLISHMENTS

- nifH mRNA appears 100–120 minutes and disappears 6–9 hrs after shift to Nitrogen-fixing conditions
- Nitrogenase appears 100–120 minutes and remains through 18 hrs after shift to Nitrogen-fixing conditions
- 296 bp probe to nifH generated by PCR and binds to nifH mRNA extracted from Nitrogen-fixing bacteria

SIGNIFICANCE

- Marine Nitrogen-fixing bacteria respond rapidly to Nitrogen-fixing conditions
- In situ hybridization techniques must use both nucleic acid probes and antisera to resolve Nitrogen-fixing bacteria from mixed populations