

AD-A191 861

ECOLOGY AND MOLECULAR GENETIC STUDIES OF MARINE  
BACTERIA(U) MARYLAND UNIV COLLEGE PARK DEPT OF  
MICROBIOLOGY R R COLWELL ET AL. 31 JAN 88

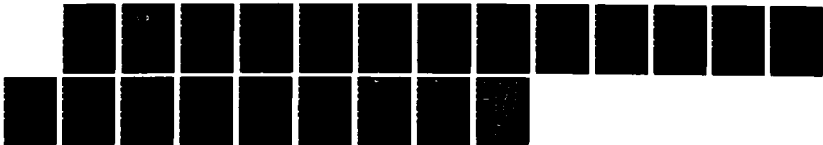
1/1

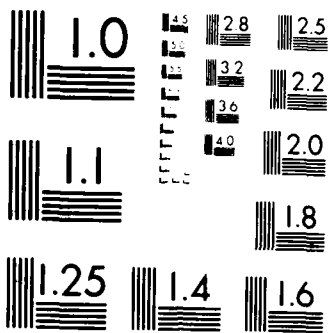
UNCLASSIFIED

N00014-86-K-0197

F/G 8/1

NL





DTIC FILE COPY

DISTRIBUTION STATEMENT A

Approved for public release; Distribution Unlimited

AD-A191 861 REPORT DOCUMENTATION PAGE

2a SECURITY CLASSIFICATION AUTHORITY NA		1b. RESTRICTIVE MARKINGS NA	
2b DECLASSIFICATION/DOWNGRADING SCHEDULE NA FEB 24 1988		3. DISTRIBUTION/AVAILABILITY OF REPORT Distribution Unlimited	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) NA		5. MONITORING ORGANIZATION REPORT NUMBER(S) NA	
6a. NAME OF PERFORMING ORGANIZATION University of Maryland	6b. OFFICE SYMBOL (if applicable) NA	7a. NAME OF MONITORING ORGANIZATION Office of Naval Research	
6c. ADDRESS (City, State, and ZIP Code) Department of Microbiology College Park, MD 20742		7b. ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research	8b. OFFICE SYMBOL (if applicable) ONR	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-86-K-0197	
8c. ADDRESS (City, State, and ZIP Code) 800 N. Quincy Street Arlington, VA 22217-5000		10. SOURCE OF FUNDING NUMBERS	
		PROGRAM ELEMENT NO 61153N	PROJECT NO. RR04106
		TASK NO	WORK UNIT ACCESSION NO
11 TITLE (Include Security Classification) Ecology and Molecular Genetic Studies of Marine Bacteria			
12 PERSONAL AUTHOR(S) R.R. Colwell, W. Straube, C. Somerville, L. Palmer, C. Pillidge, I. Knight, B. Ortiz-Conde, S. Steven			
13a TYPE OF REPORT Annual	13b. TIME COVERED FROM 6/87 TO 2/88	14 DATE OF REPORT (Year, Month, Day) 1-31-88	15. PAGE COUNT
16 SUPPLEMENTARY NOTATION			
17 COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	
19 ABSTRACT (Continue on reverse if necessary and identify by block number) Research carried out includes sequencing 5S rRNA from <i>Vibrio</i> spp., primarily of marine origin. Analysis of these sequences revealed phylogenetic relationship between these and other members of the family <i>Vibrionaceae</i> . DNA/DNA hybridizations of the <i>Vibrionaceae</i> are also in progress and results will be correlated with 5S rRNA data. In addition, cloning of the 5S rRNA genes from <i>Vibrio natriegens</i> and <i>Vibrio parahaemolyticus</i> into <i>E. coli</i> is underway. Hybridization of apparently non-luminous <i>Vibrio cholerae</i> strains with lux gene probes indicates the presence of luminescence genes which are not being expressed. Sequencing of the chitinase operon of <i>Vibrio vulnificus</i> has continued and is expected to be completed by midyear of 1988. The cloning of chitinase genes of the obligately barophilic bacterium BNL-1 is in progress. Fragments and oligomers derived from chitinolytic determinants of chitinase positive organisms are being developed as probes for estuarine and marine bacteria. The development of a method for simple, rapid isolation of total procaryotic DNA from aquatic habitats has been accomplished. Another method under development will address the problem of monitoring low copy number genes in bacteria in the environ-			
20 DISTRIBUTION/AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION ment.	
22a NAME OF RESPONSIBLE INDIVIDUAL		22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL



still can be considered separate species. The 5S rRNA sequences of V. ordalii ATCC 33509 and V. tubiashii ATCC 19105 were also determined, and it is concluded from the data (Pillidge and Colwell, 1988) that both species belong to the recently described genus Listonella (MacDonell and Colwell, 1985). A clear definition of the genus, based on 5S rRNA sequence comparisons and numerical taxonomy, is being done to determine the position of the genus within the family Vibrionaceae.

Colwell and MacDonell (1988) reported the existence of a Vibrio sp. from Antarctic rock samples. This finding was based on analysis of 5S rRNA extracted directly from the rock. In addition, a bacterium was isolated by enrichment of crushed rock samples and its 5S rRNA was sequenced. Analysis of the sequence indicates it is a Bacillus sp. Vibrio sp. were also isolated and these are being sequenced to assess similarity to the 5S rRNA sequence that was obtained by direct extraction from the rock samples.

Exciting results were obtained when determination of the 5S rRNA sequence of a common commensal of oyster larvae was accomplished. This microorganism, designated LST, was identified as a member of the genus Alteromonas (Weiner et al., 1988), based on available taxonomic data. Sequence analysis of the 5S rRNA suggests that LST, along with related strains identified by other investigators as Alteromonas belongs to the genus Shewanella. This genus, proposed by MacDonell and Colwell (1985), comprises certain members of Alteromonas, based on 5S rRNA sequence data. The 5S rRNA sequence obtained from LST further suggests that LST

is related to commensal bacteria of the deep-sea hydrothermal vent bivalve Calyptogena, as well as to bacterial commensals of the American clam Solemya and the marine diatom Odontella. The implication of these intriguing and unexpected findings is that a phylogenetically related set of bacteria is commensal to shellfish of widely different geographic distribution, including the hydrothermal vents of the deep sea.

Work involving cloning of a 5S rRNA gene from Vibrio natriegens ATCC 14048 into Escherichia coli strain in 71-18, using the plasmids pEMBL 8+ and pIBI 24 as vectors, is continuing. V. natriegens DNA may be highly methylated, which would make it a target for enzymatic degradation in E. coli, accounting for the difficulty experienced in obtaining clones of interest. The work has been expanded to include V. parahaemolyticus strain VPS 162-71, originally isolated from fish in Japan (Kaper et al., 1984). We have obtained a library of the organism constructed in E. coli using the plasmid vector pCVD 301 (Datta et al., 1984). A 5S rRNA probe was prepared and used to screen the library for 5S rDNA clones. The 5S rRNA was purified from VPS 162-71, partially digested with S1 nuclease and ligated to 32P-pCp using T4 RNA ligase. The 3' end-labelled 5S rRNA was hybridized to colony blots of the library, prepared on nylon membranes. Preliminary results indicate several possible 5S clones which are now being confirmed.

DNA/DNA homologies using the batch hydroxyapatite method (Brenner et al., 1969) are in progress. DNA from nine Vibrio spp. has been extracted in sufficient quantity and purity. In

the coming year, the first nine Vibrio DNA preparations which have been extracted will be hybridized. Eventually, DNA from eighteen additional Vibrio type strains will be extracted and hybridized. The data will be analyzed by computer, programs for which are now being written. The 5S rRNA and DNA/DNA hybridization data will be compared in order to assess the "noise" in each identification system for marine bacteria and to corroborate the phylogenetic analyses of marine bacteria.

The genes responsible for bioluminescence in Vibrio cholerae biotype albensis have been cloned into the suicide vector pEcoR251. Escherichia coli transformed with this construct acquires the bioluminescent phenotype without the addition of aldehyde, suggesting that all of the genes required for light production are present.

To determine genetic relatedness between V. cholerae biotype albensis and two other bioluminescent species, Vibrio harveyi and Vibrio fischeri, the genes which code for the luciferase enzyme will be sequenced by the enzymatic dideoxy method of Sanger (Sanger et al., 1977). A detailed restriction map of the cloned fragment has been obtained for this purpose. This fragment has been subcloned into three different reading frames of pUC plasmids (pUC8, pUC9, pUC19) using five restriction enzymes found in the multiple cloning sites of these plasmids. The purpose of the subcloning is to obtain the genes which code for the luciferase enzyme in the reading frame required for expression. Two sets of experiments are now underway to determine expression of these

genes. In Vibrio harveyi it has been found that clones containing the luciferase genes luminesce in the presence of exogenous aldehyde (Hastings et al., 1969). Screening of the subclones has not yielded a clone which is luminescent upon addition of aldehyde. This experiment will be repeated using different parameters. Expression of the luciferase genes will also be investigated using an in vitro transcription/translation assay.

The study of environmentally important genes, as well as the tracking of genetically engineered microorganisms (GEMS), often necessitates the ability to monitor the presence of genes in a particular habitat. When studying the genetic fluxes of aquatic habitats, the process of monitoring a specific nucleic acid sequence can be complicated by the species diversity of the habitat, the relative abundance of sequences in the total DNA pool, and the sheer volume of the habitat. We have approached this rather broad and complex problem through three specific areas of research; i) the isolation and characterization of an environmentally important bacterial operon (the chitinase operon of Vibrio vulnificus) as a model system, ii) the development of a method to facilitate the isolation of total procaryotic DNA from water samples, and iii) the development of a system to enhance the sensitivity of nucleic acid probes.

Our initial work with the chitinase operon of V. vulnificus has been reported (Wortman et al., 1986). Since then, research on this operon has largely centered around determination of the complete DNA sequence. We expect the completion of the sequence analysis early in 1988.

In December, 1987, we isolated several thousand bacterial colonies from Chesapeake Bay sediments. We have selected 450 isolates from this group to be probed with fragments of the chitinase operon. The results of the probe experimentation will be compared to results obtained by culturing the isolates on emulsified chitin overlays, and to rapid chemical assays for chitinolytic enzymes (Wortman et al, 1986; O'Brien and Colwell, 1987).

We have recently completed the development of a method for the simple and rapid isolation of total procaryotic DNA from aquatic habitats. The methods are outlined in the appended abstract (Somerville et al, 1988) which has been accepted for presentation at the First International Conference on the Release of Genetically Engineered Microorganisms (REGEM 1). The use of ultrafiltration to concentrate bacterial cells from water samples was chosen due to the difficulty in concentrating "starved" cells via centrifugation (unpublished observation). We have found the method to be simple, relatively rapid, and readily adaptable to shipboard use. We are now in the process of exploring a variety of potential applications for the method, including shotgun cloning of environmental DNA, biomass estimation, enrichment of specific organisms in environmental samples and the tracking of specific genes in aquatic environments.

The method has been used to concentrate 10 to 50 ml samples of hydrothermal vent fluids from both Juan de Fuca (Atlantis II cruise) and 21° N. (provided by Dr. J. Deming) vent sites. Preliminary fluorospectrographic analysis of crude DNA extracts

(Paul and Myers, 1982) of some samples show the presence of significant concentrations of DNA in relatively pure 'black smoker' fluid. This finding, when confirmed, provides important new evidence for the presence of autochthonous bacteria in these environments. We are working to improve our ability to quantify DNA present in relatively unpurified extracts, and to correlate the amount of DNA detected with bacterial numbers. Once the technique has been refined, the remaining black smoker fluid samples will be analyzed.

Chesapeake Bay water was sampled in December, 1987, using our ultrafiltration technique and a method for enrichment culture of enteric bacteria. Results indicate that ultrafiltration, using Sterivex filters, combined with enrichment culture within the filter chamber, is an inexpensive, simple method for culturing bacteria from liter quantities of water. Nucleic acids are also being extracted from Chesapeake Bay water samples and will be probed with synthesized oligomers specific for the chitinase operon of Vibrio vulnificus, using the polymerization-enhanced technique described below. This data will be compared with the results of the probing of the sediment isolates.

The second method under development in the laboratory is intended to address the problems of monitoring genes in the environment which are present in low copy numbers. The method involves probing for the presence of the gene(s) with specific oligomers, and enhancing the probe signal via the polymerization of radiolabeled DNA. As above, the appended abstract (Somerville

et al, 1988a) has been accepted for presentation at REGEM 1. Experimentation to date indicates that an acceptable signal to noise ratio can be attained by 3'-OH blocking procedures established in our laboratory. Current experiments are intended to optimize the polymerization reactions, and to compare signal sensitivity to available technology.

#### Plans for next year

We plan to conduct the following studies:

1. Resolution of the systematics of bacterial commensals of marine invertebrate organisms, beginning with the confirmed determination of the relationship of LST to Shewanella spp.
2. Further analysis of antarctic rock samples for Vibrio spp. and related microorganisms, and their relationship to species of the Vibrionaceae.
3. Determination of the 5S rRNA sequences of newly described Vibrio spp. e.g. V. hollisae, V. furnissi and V. orientalis, in order to expand knowledge of the Vibrionaceae, a family widely distributed in the world oceans.
4. Continue development of methods for extracting DNA from marine water samples in a form which can be used for DNA probe analysis. These probes will include those derived from the chitinase, luciferase and toxin genes cloned thus far, as well as synthetic oligonucleotide probes.

5. Complete sequence analysis of the chi operon in pATW 501 and extrapolate the methodology to cloning of the urease gene.
6. The cloned 5S rRNA gene(s) will be isolated, mapped using restriction endonucleases, and sequenced using the dideoxy method of Sanger (11). The 5S rDNA will then be mutated using site-directed oligonucleotide mutogenesis.
7. Establish a phylogeny based upon DNA/DNA homologies for the family Vibrionaceae and compare it with that obtained from 5S rRNA sequences by computer, using Principal Components Analysis and computer graphics for analysis of comparability of the methods.

#### LITERATURE CITED

- Brenner, D.J., G.R. Fanning, A.V. Rake, and K.E. Johnson. 1969. Batch procedure for thermal election of DNA from hydroxy apatite. *Anal. Biochem.* 28:447-459.
- Colwell, R.R. and M.T. MacDonell. 1988. Identification of an antarctic endolithic microorganism by 5S rRNA sequence analysis. *Science*. (In press).
- Datta, R.R., J.B. Kaper, and A.M. MacQuillan. 1984. Shuttle cloning vectors for the marine bacterium Vibrio parahaemolyticus. *J. Bact.* 160:808-811.
- Hastings, J.W., K. Weber, J. Friedland, A. Eberhard, G.W. Mitchell, and A. Gunsalus. 1969. Structurally distinct bacterial luciferases. *Biochem.* 8:4681-4689.
- Kaper, J.B., R.K. Campen, R.J. Seidler, M.M. Baldini & S. Falkow. 1984. Cloning of the thermostable direct or Kanagawa phenomenon-associated hemolysin of Vibrio parahaemolyticus. *Inf. and Immun.* 45(1):290-292.
- MacDonell, M.T. and R.R. Colwell. 1985. Phylogeny of the Vibrionaceae and recommendation for two new genera, Listonella and Shewanella. *Syst. Appl. Microbiol.* 6:171-182.
- MacDonell, M.T., J. M. Hansen and B.A. Ortiz-Conde. 1987. Isolation, purification and enzymatic sequencing of RNA. *Methods in*

Microbiology, Chapter 10, R.R. Colwell and R. Grigorova (eds.)  
Academic Press. pp. 357-404.

O'Brien, M.O. and R.R. Colwell. 1987. A rapid test for chitinase activity that uses 4-methylumbelliferyl-N-acetyl-B-D-glucosamide. *Appl. Environ. Microbol.* 53:1718-1720.

Paul, J.H., and B. Myers. 1982. Fluorometric determination of DNA in aquatic microorganisms by use of Hoechst 33258. *43*:1393-1399.

Pillidge, C.J., and R.R. Colwell. 1988. Nucleotide sequence of the 5S rRNA from *Listonella* (*Vibrio*) *ordalii* ATCC 33509 and *Listonella* (*Vibrio*) *tubiashii* ATCC 19105. *Nucl. Acids Res.*

Sanger, F., S. Nicklan and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA*, *74*(12):5463-5467.

Somerville, C.C., I.T. Knight, W.L. Straube and R.R. Colwell. 1988. A simple, rapid method for the direct isolation of nucleic acids from the aquatic environment. REGEM 1. Cardiff, Wales. Abstract.

Somerville, C.C., I.T. Knight, W.L. Straube, and R.R. Colwell. 1988a. Probe-directed, polymerization-enhanced detection of specific gene sequences in the environment. REGEM 1. Cardiff, Wales. Abstract.

Weiner, R.M., V.E. Coyne, P. Brayton, P. West, S.F. Raiken. Alteromonas colwelliana sp. nov., a new isolate from oyster habitats. Int. J. Syst. Bact. (submitted).

Wortman, A.T., C.C. Somerville, and R.R. Colwell. 1986. Chitinase determinants of Vibrio vulnificus: Gene cloning and applications of a chitinase probe. Appl. Environ. Microbiol. 52:142-145.

Publications supported by:

ONR Grant #N00014-81-K-0638

Pillidge, C.J. and R.R. Colwell. 1988. Nucleotide sequence of the 5S rRNA from Listonella (Vibrio) ordalii ATCC 33509 and Listonella (Vibrio) tubiashii ATCC 19105. Submitted to Nucleic Acids Research.

Somerville, C.C., I.T. Knight, W.L. Straube and R.R. Colwell. 1988. Probe-directed, polymerization-enhanced detection of specific gene sequences in the environment. Abstract. REGEM I, Cardiff, Wales.

Somerville, C.C., I.T. Knight, W.L. Straube and R.R. Colwell. 1988. A simple, rapid method for the direct isolation of nucleic acids from the aquatic environment. Abstract. REGEM I, Cardiff, Wales.

Pillidge, C.J., M.T. MacDonell and R.R. Colwell. 1987. Nucleotide sequence of the 5S rRNA from Listonella (Vibrio) aestuarianus ATCC 35048. Nucleic Acids Res. 15(4):1879.

Lau, P.O., B. Debrunner-Vossbrinch, B. Dunn, K. Miotto, M.T. MacDonell, D.M. Rollins, C.J. Pillidge, R.B. Hespell, R.R. Colwell, M.L. Sogin and G.E. Fox. 1987. Phylogenetic diversity and position of the genus Campylobacter. Sys. & Appl. Microbiol. 9:231-238.

Roszak, D., R.R. Colwell. 1987. Metabolic activity of bacterial cells enumerated by direct viable count. *Appl. & Environ. Microbiol.* 53:2889-2893.

Kogure, K., U. Simidu, N. Tage and R.R. Colwell. 1987. Correlation of direct viable counts with heterotrophic activity for marine bacteria. *Appl. & Environ. Microbiol.* 53:2332-2337.

Roszak, D.B. and R.R. Colwell. 1987. Survival strategies of bacteria in the natural environment. *Microbiol. Rev.* 51:365-379.

Grimes, D.J., C.C. Somerville, W.L. Straube, D.B. Roszak, B.A. Ortiz-Conde, M.T. MacDonell and R.R. Colwell. 1986. Plasmid mobility in the ocean environment. Vol. 10, *Aquatic toxicology & Environmental Fate*, ASTM, Phila., PA. In press.

MacDonell, M.T., B.A. Ortiz-Conde, G.A. Last and R.R. Colwell. 1986. Distribution of mutations in Gram negative eubacterial 5S rRNAs and significance for sequence analysis. *J. Microbiol. Methods*. In press.

MacDonell, M.T., E. Russek-Cohen, D.G. Swartz and R.R. Colwell. 1986. A modified principal components approach to RNA sequence analysis. *J. Microbiol. Meth.* In press.

MacDonell, M.T., S.C. Morris, B.A. Ortiz-Conde, C.J. Pillidge and R.R. Colwell. 1986. Application of ion exchange HPLC to

the purification of 5S rRNAs suitable for sequence analysis.  
J. Chromatography. 363:438-443.

West, P.A., P.R. Brayton, T.N. Bryant and R.R. Colwell. 1986.  
Numerical taxonomy of Vibrios isolated from aquatic environ-  
ments. Int. J. Sys. Bacteriol. 36:531-543.

Okpokwasili, G.C., C.C. Somerville, M. Sullivan, D.J. Grimes and  
R.R. Colwell. 1986. Plasmid mediated degradation of hydro-  
carbons in estuarine bacteria. Oil Petrochem. Pollut.  
3:117-129.

MacDonell, M.T. and R.R. Colwell. 1985. Nuclease S<sub>1</sub> analysis of  
eubacterial 5S rRNA secondary structure. J. Mol. Evol.  
22:237-242.

MacDonell, M.T., D.G. Swartz, B.A. Ortiz-Conde, G.A. Last and  
R.R. Colwell. 1986. Ribosomal RNA phylogenies for the  
vibrio-enteric group of eubacteria. Microbiol. Sci. 3:172-  
178.

Grimes, D.J., P.R. Brayton, P.A. West, F.L. Singleton and R.R.  
Colwell. 1986. The probabilistic identification of Vibrio  
spp. isolated from surface seawater with special reference  
to Vibrio campbellii. Lett. Appl. Microbiol. 2:93-95.

Colwell, R.R., M.T. MacDonell and J. DeLey. 1986. Proposal to  
recognize family Aeromonadaceae fam. nov. Int. J. Syst.  
Bacteriol. 36:473-477.

- Grimes, D.J. and R.R. Colwell. 1986. Viability and virulence of Escherichia coli suspended by membrane chamber in semitropical ocean water. FEMS Microbiol. Lett. 34:161-165.
- Wortman, A.T., C.C. Somerville and R.R. Colwell. 1986. The chitinase determinants of Vibrio vulnificus: Gene cloning and the applications of a chitinase probe. Appl. Environ. Microbiol. 52:142-145.
- MacDonell, M.T. and R.R. Colwell. 1985. Nuclease S<sub>1</sub> analysis of eubacterial 5S rRNA and secondary structure. J. Mol. Evol. 22:104-108.
- Brayton, P.R., R.B. Bode, R.R. Colwell, M.T. MacDonell, H.L. Hall, D.J. Grimes, P.A. West and T.N. Bryant. 1986. Vibrio cincinnatiensis, sp. nov., a new human pathogen. J. Clin. Microbiol. 23:104-108.
- MacDonell, M.T. and R.R. Colwell. 1985. The phylogeny of the Vibrionaceae, and recommendation for two new genera, Listonella and Shewanella. Syst. Appl. Microbiol. 6:171-182.
- Colwell, R.R., P.R. Brayton, D.J. Grimes, D.R. Roszak, S.A. Huq and L.M. Palmer. 1985. Viable, but nonculturable Vibrio cholerae and related pathogens in the environment: Implications for release of genetically engineered microorganisms. Bio/Tech. 3:817-820.
- Elliot, E.L. and R.R. Colwell. 1985. Indicator organisms for estuarine and marine waters. FEMS Microbiol. Rev. 132:61-79.

MacDonell, M.T. and R.R. Colwell. 1985. The nucleotide sequence of 5S ribosomal RNA from Vibrio marinus. Microbiol. Sci. 9:229-231.

MacDonell, M.T. and R.R. Colwell. 1984. A microcomputer program for the computation of free energy of the secondary structure of RNA oligomers by Ninio's rules. J. Microbiol. Meth. 3:33-42.

MacDonell, M.T. and R.R. Colwell. 1984. Nucleotide base sequence of vibronaceae 5S rRNA. FEBS Lett. 175:183-188.

MacDonell, M.T. and R.R. Colwell. 1984. Identical 5S rRNA nucleotide sequence of Vibrio cholerae strains representing temporal, geographical, and ecological diversity. Appl. Environ. Microbiol. 48:119-121.

MacDonell, M.T. and R.R. Colwell. 1984. Dot matrix analysis of nucleic acid sequences: An interactive microcomputer program. Mol. Biol. Eval. 3:247-254.



St. David's Hall, Cardiff, Wales, U.K. April 1988

The title of the subject category (See Final Circular for lists) into which this poster best falls is:

LECTURE Survival, Persistence and Detection

You must send:

- (i) The original typed copy of this Abstract Form
- (ii) Two good photocopies of the typed Abstract Form
- (iii) One stamped self-addressed postcard not smaller than 8cm x 15cm carrying at the top the title of the paper and author(s) name(s). IMPORTANT: For mailing purposes in connection with poster submissions, please use your best mailing address for November 1987 - April 1988.

TO ARRIVE NOT LATER THAN 1st NOVEMBER 1987

**PREPARATION OF ABSTRACTS**

YOUR ABSTRACT WILL BE REPRODUCED PHOTOGRAPHICALLY AND APPEAR IN THE ABSTRACT BOOK EXACTLY AS TYPED.

BECAUSE YOUR ABSTRACT WILL BE REPRODUCED PHOTOGRAPHICALLY YOU MUST USE THIS FORM, AND NOT A PHOTOCOPY OF IT, TO PRODUCE THE ORIGINAL TYPED COPY.

Use of an electric typewriter with a good, black ribbon is advised. If possible, use a sans serif type.

Any hand-written symbols must be drawn in black ink. If corrections are necessary, use correction fluid not an eraser.

Abstracts should be informative and representative of the content of the paper. They should summarise the results to be presented and the conclusions to be drawn. Avoid phrases such as "Data will be presented" and "Results will be discussed."

The Organizing Committee may request that poorly presented abstracts are re-typed by the author.

All abstracts must be in English.

Type your abstract within the box marked on the Abstract Form using the layout shown in the example and as described below:

Type single spaced as close to the boundaries of the box as possible. Do not leave a margin at the top or on the left-hand side.

Commence at the top left-hand corner with the title of the abstract.

On the next line type IN CAPITAL LETTERS the initials and family name (surname) of the presenting author and co-author(s). The name of the *presenting author* should be underlined. Do not give titles or qualifications of the author(s).

Continue on the same line with the name and short addresses of the Institution(s) where the research was carried out. Give the city (state) and country but do not include a post or zip code. Indicate author's address with superscript numerals after surnames and addresses.

Leave one blank line and begin the abstract, typing single-spaced.

Instructions for Abstract Preparation  
 R.C.W. BERKELEY<sup>1</sup> and H. SMITH<sup>2</sup> Department of Microbiology, University of Bristol, Bristol, UK<sup>1</sup> and Department of Microbiology, University of Birmingham, Birmingham, UK<sup>2</sup>  
 This illustrates the style in which abstracts should be typed. The hatched area is for use by the Organizing Committee and will contain an abstract number.

Please do not type in the hatched area

A Simple, Rapid Method for the Direct Isolation of Nucleic Acids from the Aquatic Environment.  
 C.C.SOMERVILLE, I.T.KNIGHT, W.L.STRAUBE and R.R.COLWELL. Department of Microbiology, University of Maryland, College Park, MD, USA.

The direct isolation of nucleic acids from the environment may be useful in several respects. These include, the estimation of total biomass, detection of specific organisms (e.g. GEMS) and genes, quantitation of gene expression, estimations of species diversity and cloning applications. We have developed a method which facilitates the concentration of microorganisms from aquatic samples and the extraction of their nucleic acids. Liter quantities of sample are first blended to dissociate organisms from particulates, passed through a series of prefilters and concentrated on a single, cylindrical filter membrane (Millipore SVGS01015). Cell lysis and proteolysis are carried out within the filter housing. Crude, high molecular weight nucleic acid solutions are then drawn off the filter. These solutions can be immediately analyzed, concentrated, or purified depending on intended application. The method is both simple and economical and provides DNA/RNA of concentration and purity equal to or exceeding that of published methods.

DO NOT FOLD ACROSS THE BOX

The title of the subject category (See Final Circular for lists) into which this poster best falls is:

LECTURE Survival, Persistence and Detection

You must send:

- (i) The original typed copy of this Abstract Form
- (ii) Two good photocopies of the typed Abstract Form
- (iii) One stamped self-addressed postcard not smaller than 8cm x 15cm carrying at the top the title of the paper and author(s) name(s). **IMPORTANT:** For mailing purposes in connection with poster submissions, please use your best mailing address for November 1987 - April 1988.

TO ARRIVE NOT LATER THAN 1st NOVEMBER 1987

**PREPARATION OF ABSTRACTS**

YOUR ABSTRACT WILL BE REPRODUCED PHOTOGRAPHICALLY AND APPEAR IN THE ABSTRACT BOOK EXACTLY AS TYPED.

BECAUSE YOUR ABSTRACT WILL BE REPRODUCED PHOTOGRAPHICALLY YOU MUST USE THIS FORM, AND NOT A PHOTOCOPY OF IT, TO PRODUCE THE ORIGINAL TYPED COPY.

Use of an electric typewriter with a good, black ribbon is advised. If possible, use a sans serif type.

Any hand-written symbols must be drawn in black ink. If corrections are necessary, use correction fluid not an eraser.

Abstracts should be informative and representative of the content of the paper. They should summarise the results to be presented and the conclusions to be drawn. Avoid phrases such as "Data will be presented" and "Results will be discussed".

The Organizing Committee may request that poorly presented abstracts are re-typed by the author.

All abstracts must be in English.

Type your abstract within the box marked on the Abstract Form using the layout shown in the example and as described below.

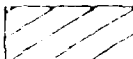
Type single spaced as close to the boundaries of the box as possible. Do not leave a margin at the top or on the left-hand side.

Commence at the top left-hand corner with the title of the abstract.

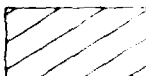
On the next line type IN CAPITAL LETTERS the initials and family name (surname) of the presenting author and co-author(s). The name of the presenting author should be underlined. Do not give titles or qualifications of the author(s).

Continue on the same line with the name and short addresses of the institution(s) where the research was carried out. Give the city (state) and country but do not include a post or zip code. Indicate author's address with superscript numerals after surnames and addresses.

Leave one blank line and begin the abstract, typing single-spaced.

 Instructions for Abstract Preparation  
 R.C.W. BERKELEY<sup>1</sup> and H. SMITH<sup>2</sup> Department of Microbiology, University of Bristol, Bristol, UK<sup>1</sup> and Department of Microbiology, University of Birmingham, Birmingham, UK<sup>2</sup>  
 This illustrates the style in which abstracts should be typed. The hatched area is for use by the Organizing Committee and will contain an abstract number.

Please do not type in the hatched area

 Probe-Directed, Polymerization-Enhanced Detection of Specific Gene Sequences in the Environment.  
 C.C.SOMERVILLE, I.T.KNIGHT, W.L.STRAUBE and R.R.COLWELL. Department of Microbiology, University of Maryland, College Park, MD.

The ability to detect genetically engineered microorganisms (GEMs) in the environment may be considered a necessary prerequisite for their release. In some cases such detection will be complicated by a rapid dilution of the GEMs, both by the shear volume of the habitat and its species diversity. While antibody-mediated detection methods may be sensitive and specific for the original organism released, they do not directly monitor the fate of the engineered gene. We have devised a method to enhance the sensitivity of classic gene-probing technology based upon the use of specific hybridization probes as primers for chain elongation. Target DNA is first treated with DNA pol I and dideoxy-nucleotides to eliminate free 3'-OH residues. Unlabeled probe DNA is then hybridized to target and serves as primer for extensive chain elongation with labeled deoxy-nucleotide(s). Specificity is determined by probe hybridization, while enhanced sensitivity is provided by polymerization into adjacent genes. With this method it should be possible to increase sensitivity by two orders of magnitude or more over conventional methods.

DO NOT FOLD ACROSS THE BOX

END

DATE

FILMED

6-88

DTIC