INTERNATIONAL CENTRE FOR DIARRHOEAL DISEASE RESEARCH, BANGLADESH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone : 871751-60, Telex : 675612 ICDD BJ
Fax : 880-2-883116, 886050, 871568, 871686, Cable : Cholera Dhaka

CENTRE
FOR HEALTH AND
POPULATION RESEARCH

MEMORANDUM

16th August 2001

To : Dr. Md. Sirajul Islam
Laboratory Sciences Division

From: *David A Sack, M D*
Chairman, Research Review Committee (RRC)

Sub : **Protocol # 2001-018**

Thank you for your protocol # 2001-018 entitled "Environmental resistance of *V. cholerae*" which the RRC considered in its meeting held on 13th August 2001. After review and discussion, the Committee made the following observations on your protocol:

a) The Investigators should provide some background information of the studies including the NIH-funded study, already conducted at different ponds at Matlab and Chandpur areas (salinity, P^H, water quality, algae concentration, persistence of *Vibrio cholerae* in different seasons etc.)

b) The project summary should contain a brief statement of the problem and study sites.

c) Figure # 4 (p 9) should be re-numbered as 1 since this is the first figure in the protocol. The rest of the figures should also be sequentially numbered.

d) The criteria for selection of one pond at Matlab and the other at Chandpur, be provided.

e) Other costs (budget) for US$73,894 need to be specified.

You are, therefore, advised to modify the protocol incorporating the above observations and submit the modified version for consideration of the Chair.

Thank you.

Copy: Acting Head
Laboratory Sciences Division

FOR OFFICE USE ONLY

RRC Approval: Yes/ No Date:

ERC Approval: Yes/No Date:

AEEC Approval: Yes/No Date:

RESEARCH PROTOCOL

Protocol No.: 2001-018

Project Title: **ENVIRONMENTAL PERSISTENCE OF *Vibrio cholerae***

Theme: ***(Check all that apply)***

- ☐ Nutrition
- ☐ Emerging and Re-emerging Infectious Diseases
- ☐ Population Dynamics
- ☐ Reproductive Health
- ☐ Vaccine evaluation
- ☑ Environmental Health
- ☐ Health Services
- ☐ Child Health
- ☐ Clinical Case Management
- ☐ Social and Behavioural Sciences

Key words: Environment, *V. cholerae*, Microarray, Biocomplexity, Zooplankton, Phytoplankton

Principal Investigator(s): Dr. Md. Sirajul Islam (Local) **Division: LSD** **Phone:** 8811751-60, Ext.2407
Professor Ronald K. Taylor, USA

Address: Environmental Microbiology Laboratory, LSD ICDDR,B **Email: sislam@icddrb.org**

Co-Principal Investigator(s):

Co-Investigator(s): Dr. Anwarul Huq, USA
Dr. Deborah Chiavelli, USA

Student Investigator/Intern:

Collaborating Institute(s): Dartmouth Medical School, USA

Population: Inclusion of special groups (*Check all that apply*):

Gender
- ☐ Male
- ☐ Females

Age
- ☐ 0 – 5 years
- ☐ 5 – 9 years
- ☐ 10 – 19 years
- ☐ 20 +
- ☐ > 65

- ☐ Pregnant Women
- ☐ Fetuses
- ☐ Prisoners
- ☐ Destitutes
- ☐ Service providers
- ☐ Cognitively Impaired
  - ☐ CSW
- ☐ Others (specify: Not Applicable)
- ☐ Animal

Project / study Site (*Check all the apply*):

- ☐ Dhaka Hospital
- ☐ Matlab Hospital
- ☑ Matlab DSS area
- ☐ Matlab non-DSS area
- ☐ Mirzapur
- ☐ Dhaka Community
- ☐ Chakaria
- ☐ Abhoynagar
- ☐ Mirsarai
- ☐ Patyia
- ☐ Other areas in Bangladesh: Dhaka, Chandpur
- ☐ Outside Bangladesh
  name of country: ____________
- ☐ Multi centre trial
  (Name other countries involved)
  ____

Type of Study *(Check all that apply)*:

- ❒ Case Control study
- ❒ Community based trial / intervention
- ❒ Program Project (Umbrella)
- ❒ Secondary Data Analysis
- ❒ Clinical Trial (Hospital/Clinic)
- ❒ Family follow-up study
- ❒ Cross sectional survey
- ❒ Longitudinal Study (cohort or follow-up)
- ❒ Record Review
- ❒ Prophylactic trial
- ❒ Surveillance / monitoring
- ☑ Others

Targeted Population *(Check all that apply)*: Not Applicable

- ❒ No ethnic selection (Bangladeshi)
- ❒ Bangalee
- ❒ Tribal groups
- ❒ Expatriates
- ❒ Immigrants
  - ❒ Refugee

Consent Process *(Check all that apply)*: Not Applicable

- ❒ Written
- ❒ Oral
- ❒ None
- ❒ Bengali language
- ❒ English language

Proposed Sample size: Not Applicable

Total sample size: ______________ ❒

Sub-group ______________ ❒ ______________ ❒

______________ ❒ ______________ ❒

Determination of Risk: Does the Research Involve *(Check all that apply)*: Not Applicable

- ❒ Human exposure to radioactive agents?
- ❒ Fetal tissue or abortus?
- ❒ Investigational new device?
  (specify______________)
- ❒ Existing data available from Co-investigator
- ❒ Human exposure to infectious agents?
- ❒ Investigational new drug
- ❒ Existing data available via public archives/source
- ❒ Pathological or diagnostic clinical specimen only
- ❒ Observation of public behaviour
- ❒ New treatment regime

Yes/No

❒ ☑ Is the information recorded in such a manner that subjects can be identified from information provided directly or through identifiers linked to the subjects?

❒ ☑ Does the research deal with sensitive aspects of the subject's behaviour; sexual behaviour, alcohol use or illegal conduct such as drug use?

Could the information recorded about the individual if it became known outside of the research:

❒ ☑ a. place the subject at risk of criminal or civil liability?

❒ ☑ b. damage the subject's financial standing, reputation or employability; social rejection, lead to stigma, divorce etc.

Do you consider this research *(Check one)*:

- ❒ greater than minimal risk
- ☑ no risk
- ❒ no more than minimal risk
- ❒ only part of the diagnostic test

*Minimal Risk is "a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests. For example, the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than the risk of doing so as a part of routine physical examination".

Yes/No

☑ ☐ Is the proposal funded?

If yes, sponsor Name: NATIONAL SCIENCE FOUNDATION, USA

Yes/No

☐ ☐ Is the proposal being submitted for funding ? Not Applicable

If yes, name of funding agency: (1) ____________________

(2) ____________________

Do any of the participating investigators and/or their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

IF YES, submit a written statement of disclosure to the Director.

Dates of Proposed Period of Support — **Cost Required for the Budget Period ($)**

(Day, Month, Year - DD/MM/YY)	***a.***	***Ist Year***	***2nd Year***	***3rd Year***	***Other years***
Beginning date: September, 2001		**81,790**	**82,806**	**86,276**	________
End date: August, 2004	***b.***	***Direct Cost :*** US $ 250,872		***Total Cost :*** US $ 250,872	

Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

Dr. G.B. Nair
Name of the Division Director

[signature]
Signature

August 6, 2001
Date of Approval

Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

Signature of PI M.S. Islam

Date: 05.08.2001

Name of Contact Person (if applicable)
DR. MD. SIRAJUL ISLAM

Table of Contents

	Page Numbers
Face Page	1-3
Project Summary	5
Description of the Research Project	6-29
Hypothesis to be tested	6-7
Specific Aims	7-9
Background of the Project Including Preliminary Observations	10-13
Research Design and Methods	14-18
Facilities Available	19
Data Analysis	20
Ethical Assurance for Protection of Human Rights	21
Use of Animals	21
Literature Cited	22-27
Dissemination and Use of Findings	28
Collaborative Arrangements	29
Biography of the Investigators	30-36
Detalied Budget	39-42
Budget Justifications	43
Other Support	44-46
Ethical Assurance : Protection of Human Rights	
Appendix	
Consent Forms in English	
Consent Forms in Bangla	

Check here if appendix is included

PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. (TYPE TEXT WITHIN THE SPACE PROVIDED).

Principal Investigators: **Dr. Md. Sirajul Islam (Local)**
Ronald K. Taylor, Ph.D. (USA)

Project Name: **Environmental persistence of *Vibrio cholerae***

Total Budget: US $ 250,872.00 | Beginning Date: September, 2001 | Ending Date: August, 2004

PROJECT SUMMARY:

We propose to use microarrays to facilitate whole-genome analysis in three interrelated studies:

1. A laboratory experiment to determine the temporal dynamics of gene expression, particularly the co-regulation of dormancy and attachment, under dramatically different environmental conditions in three different bacterial microhabitats
2. A laboratory experiment to determine how temperature, pH, nutrients, and salinity interact to determine gene expression, particularly for genes associated with dormancy and attachment
3. An *in situ* incubation experiment in Bangladesh that measures gene expression in bacteria as a function of seasonal variations in environmental conditions in natural systems

The proposed research will make significant contributions at the interface of ecology, genetics, microbiology, and statistics. Our three aims will provide important new information about (1) the genetic processes that instigate transfer between active and dormant states, and between attached and unattached states and (2) the extent to which these processes might be co-regulated under changing environmental conditions. The resulting improved understanding of how bacteria react to changes in environmental factors will help us to better understand bacterial roles in aquatic ecosystem processes and predict how these roles might be affected by anthropogenic processes such as land use practices, sewage treatment and climate change.

Microarrays provide a novel way to assess bacterial activity, and should elucidate mechanisms for known patterns not identifiable by other methods. For example, the metabolic costs of expressing colonization factors versus the nutritional benefit gained from associating with plankton -- and the interplay of these parameters with the dormancy status of the bacterium -- can only begin to be understood once genetic pathways for these events are established. We will also learn a great deal about the role of phytoplankton vs. zooplankton as attachment substrates for bacteria, the plasticity of attachment behaviors, the factors which lead to detachment, and the potential consequences of this attachment for ecosystem processes.

We propose to be among the first research groups to use microarray chips to measure the gene expression in organisms within their natural habitat rather than a laboratory setting. By measuring gene expression of *V. cholerae* in these systems, we will be able to determine the genetic mechanisms by which seasonal changes in environmental conditions may contribute to bacterial productivity in a tropical ecosystem.

Finally, a major benefit of using *V. cholerae* as the model environmental organism is that this research will contribute greatly to our ability to predict the timing and severity of cholera outbreaks, to understand the long term survival of *V. cholerae* in the environment, and to assess the risk of the environmental survival of *V. cholerae* if it is introduced to regions where it currently does not occur. This last point is especially important due to the potential for increase in the range of *V. cholerae* through, for example, transportation via infected humans or through the ballast water of ships (Ruiz *et al.* 2000).

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

	Name	Professional Discipline/ Specialty	Role in the Project
1.	Taylor, R.K.	Professor of Microbiology and Immunology	PI
2.	Islam, M.S.	Environmental Microbiologist	Local PI
3.	Huq, A.	Associate Professor of Microbial Ecology	Co-Investigator
4.	Chiavelli, D.A.	Zooplankton specialist	Co-Investigator

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested and the Specific Aims of the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

We have summarized our hypotheses for the temporal pattern of gene expression for bacteria that begin experiments in log-phase growth in Table 1. We expect patterns to be similar for bacteria that are initially dormant, but to start from lower levels of gene expression in all categories.

Table 1. Hypothesized temporal patterns of gene expression for bacteria initially in log phase growth for low (dashed line) vs. high (solid line) nutrient conditions in each microhabitat. For unattached bacteria, metabolism is lower in low nutrients and bacteria enter dormancy sooner; attachment factors have opposite pattern. For attached bacteria, metabolism and biofilm activity will be higher when water nutrients are higher. Because *Anabaena* are expected to be more nutritive than *Daphnia,* metabolism will be higher on *Anabaena* and metabolism and biofilm activity will decline on *Daphnia* earlier than on *Anabaena.* Detachment will have the opposite pattern as attachment. On both substrates biofilms will take time to develop resulting in an initial increase in biofilm and metabolism expression.

Functional Gene Group	Expression Over Time		
	Unattached	On *Daphnia*	On *Anabaena*
Metabolism/ Dormancy			
Attachment			
Biofilm Activity	NA		
Detachment	NA		

General: We expect that bacterial productivity and SRBs are adaptively facultative: in favorable conditions bacteria should benefit from putting more energy into productivity than into attachment behavior and biofilm elaboration.

Unattached bacteria: We hypothesize that there will be a negative correlation between gene groups that indicate high productivity and gene groups that indicate surface response behaviors (SRBs). Specifically, since the higher levels of all four environmental factors are known to be more favorable for bacterial growth, treatments with more factors at high levels should have a greater expression of productivity genes and less expression of SRB genes. However, if conditions are too poor (e.g., all factors are at low levels), unattached bacteria may become dormant (see 4).

Attached bacteria: As environmental factors improve, we expect that the relative advantage of attachment will decrease and expression of attachment-related SRBs will decline and detachment-related SRBs may increase.

Daphnia exoskeleton vs. Anabaena: We hypothesize *Anabaena* will be a better substrate for bacterial growth than chitinous exoskeletons, both because bacteria can take up organic carbon excreted by the alga (Del Giorgio and Cole 1998) and because *Anabaena* colonies contain N-fixing cells around which attached bacteria show increased growth (Islam 1999). We therefore predict greater expression of attachment factors and lower expression of detachment factors for bacteria on *Anabaena* as compared to *Daphnia* under the same environmental conditions.

Dormancy: We hypothesize that bacteria in a fully dormant state, as indicated by specific dormancy gene expression patterns, will not express SRB, and therefore will not change their microhabitat.

Hypotheses and Predictions

1) Because we will simultaneously measure physical and chemical water parameters, all Aim 1 and Aim 2 hypotheses for the three microhabitats (unattached, on *Daphnia* exoskeleton and on *Anabaena* colonies) are relevant and applicable to our field experiment.
2) We therefore expect that seasonal, inter-annual, and between-pond differences will have strong effects on gene expression by both attached and unattached bacteria.
3) We will be able to compare the field results with predictions generated from our lab experiments in Aim 1 and Aim 2 to assess the relevance of our laboratory experimental results to natural systems.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods (TYPE WITHIN LIMITS).

Aim 1: Conduct laboratory experiments that examine the long-term progression of responses by unattached and attached *V. cholerae* after transition in nutrient conditions.

Aim 2: Conduct laboratory experiments to examine genome-wide expression in response to four environmental factors by attached and unattached *V. cholerae.*

Aim 3: Field experiments to look at *in situ* seasonal effects on *V. cholerae* in freshwater ponds.

Our overall goal is to use whole-genome expression profiles of an aquatic bacterium to study the interaction of two behaviors, dormancy and attachment, which can govern bacterial productivity in aquatic ecosystems. We will utilize microarrays to assess (1) whether dormancy and attachment act independently or in concert under different environmental scenarios and (2) the degree to which these behaviors are influenced by the relative qualities of the water and planktonic surface habitats available. Our model organism, *Vibrio cholerae*, is found in planktonic communities worldwide and is representative of a large family of bacteria, the *Vibrionaceae*. Due to its medical importance as the etiological agent of cholera, *V. cholerae* has a fully sequenced and well-studied genome, enabling the use of microarray technology to study its role in aquatic ecosystems.

This proposal describes studies to achieve three major aims. Aim 1 will examine the temporal correlation in expression of genes associated with dormancy and attachment in favorable and unfavorable water conditions for bacteria exposed to three microhabitats (unattached, attached to a zooplankter, attached to a phytoplankter). Aim 2 will quantify the direct effects and interactions of four key water quality parameters (nutrients, temperature, pH, and salinity) on bacterial gene expression in the same three microhabitats. These water parameters were chosen because they influence bacterial productivity and can be strongly affected by anthropogenic activities. In Aim 3, we propose an *in situ* incubation experiment in Bangladesh to test whether patterns observed in the laboratory also occur in nature. Importantly, this study will be one of the first to use microarrays to measure the response of organisms in a natural system rather than in laboratory conditions.

This proposal results from strong interdisciplinary collaboration among scientists with expertise in ecology, microbiology, genetics, and statistics. The contributions and research facilities of our international collaborator, Dr. Sirajul Islam, are essential to providing a field context in which to test the laboratory results. Furthermore, this project will develop a group of undergraduate, graduate, postdoctoral, and faculty researchers who are familiar with each of these fields and who are able to conduct research from both interdisciplinary and multifactor perspectives.

AIM 1

The goal of this aim is to compare temporal patterns and co-regulation of metabolic behaviors, such as growth rate and dormancy, with specific surface response behaviors (SRBs) such as preparation for attachment or detachment, biofilm elaboration, or elaboration of secreted hydrolytic enzymes, by monitoring whole-genome expression of *V. cholerae*. Although both dormancy and attachment are key factors determining the role of bacteria in aquatic ecosystems, the relationship between nutrient levels and bacterial attachment and the extent and direction of metabolic change following attachment to plankton are not well characterized. Each aim 1 experiment will evaluate gene expression over 14 days in low and high nutrient treatments to determine how nutrient levels mediate the timing of dormancy and attachment. To determine how the initial bacterial condition affects the timing of their responses, we will run two sets of experiments: one starting with actively growing cells from a log-phase culture and the other with bacteria in the dormant state more typical of pelagic conditions. Each set of experiments will involve separate evaluation of bacterial gene expression in three microhabitats: unattached, attached to *Daphnia* exoskeletons, and attached to *Anabaena* colonies.

These experiments will provide valuable information on the temporal coordination of metabolic activity, dormancy, and SRBs in unattached and attached bacteria as a function of initial bacterial metabolic state and water conditions. These experiments will also increase our knowledge of the molecular basis of transition into and out of dormancy, about which little is known in spite of the ecological significance of this condition.

AIM 2

The goal of this aim is to investigate the co-regulation of metabolic activity (growth rate and dormancy) with SRBs under different environmental conditions. Laboratory experiments will determine the expression patterns of *V. cholerae* genes in combinations of four key water quality factors and three planktonic microhabitats. We have chosen to investigate temperature, nutrient availability, salinity, and pH, each of which is known to influence the physiological state of bacteria, including *V. cholerae*. These environmental factors are also of interest because they vary over a large range both within and among aquatic systems and are sensitive to the anthropogenic environmental impacts of climate change and eutrophication.

We will use a factorial experimental design to efficiently test both the direct effects and interactions of the environmental factors. Evaluating interactions in bacterial response to these factors is critical because environmental factors vary independently in natural systems. We will conduct three different microcosm experiments to examine whole-genome expression of *V. cholerae* in the same microhabitats as in Aim 1: (1) without any substrate for attachment, (2) attached to surface of a crustacean zooplankter, and (3) attached to surface of a cyanobacterium. Bacterial gene expression is expected to differ for attached and unattached individuals and between the two plankton substrates (*Daphnia* and *Anabaena*) because they present different nutritional opportunities for bacteria. As in Aim 1, the levels of the environmental factors will explore the range of conditions experienced where our focal strain of *V. cholerae* was isolated and where our field study (Aim 3) will occur, the Matlab region of Bangladesh. This work will provide insight into the relative importance of surface attachment to bacterial function over a range of environmental conditions and attachment surfaces, which will in turn help us understand the role of bacteria in different types of aquatic ecosystems. Of particular ecological importance will be information about the role of phytoplankton vs. zooplankton as bacterial microhabitats. We will also determine gene groups that can serve as optimal indicators of productivity and various types of SRB and we will focus on using these gene groups in the field study in Aim 3.

AIM 3

While laboratory experiments can help us interpret what happens in natural systems there is no way to completely replicate field conditions in the laboratory. With *in situ* incubation experiments in our field study site in the Matlab region of Bangladesh, we propose to be one of the first research groups to use microarray chips to measure the response of organisms in a natural system rather than in a laboratory setting.

We will conduct six experiments per year, timed to be coincident with the average beginning, peak, and decline of the two annual peaks in cholera outbreaks in the local human population. Since plankton abundance and cholera outbreaks are likely to be associated with periods of high bacterial productivity, these sampling times should represent transitions in gene expression associated with poor versus productive conditions. A typical seasonal progression in this location (common to many tropical freshwater systems; Fig. 4), is high nutrient input from

runoff during the rainy season, but dilution by overflow and low sunlight prevents plankton populations from developing high densities. At the beginning of the dry season sunlight increases dramatically and dilution is no longer a factor, resulting in a phytoplankton bloom followed by a zooplankton bloom. During this time, nutrients are gradually depleted from the photic zone as dead plankton and their feces sink out of the water column. A moderate amount of precipitation in March and April sometimes provide a brief period of favorable planktonic growth conditions (new nutrients, some sun) before the deluge begins again. The patterns of human cholera cases in this region, where most people get their water supply directly from ponds and lakes. have a seasonality that has been surprisingly consistent for decades. Most cases occur during early dry season/plankton bloom period, and a second, smaller outbreak sometimes occurs in March and April, but not in all years (Fig. 4, Glass *et al.* 1982, Islam *et al.* 1994a). These outbreak periods appear to mirror productive times in the ponds and thus probably times of high productivity for the bacteria, but it is not clear whether the effect on the bacteria is a result of improved water conditions, more phytoplankton surfaces, more zooplankton surfaces, or all three. By measuring gene expression of *V. cholerae* in these systems, we will be able to determine the genetic mechanisms by which seasonal changes may contribute to bacterial productivity and distribution patterns in a tropical ecosystem. Specifically, we can resolve the relative quality of our three bacterial microhabitats under varying natural conditions by observing the expression of indicator gene groups for productivity and SRB.

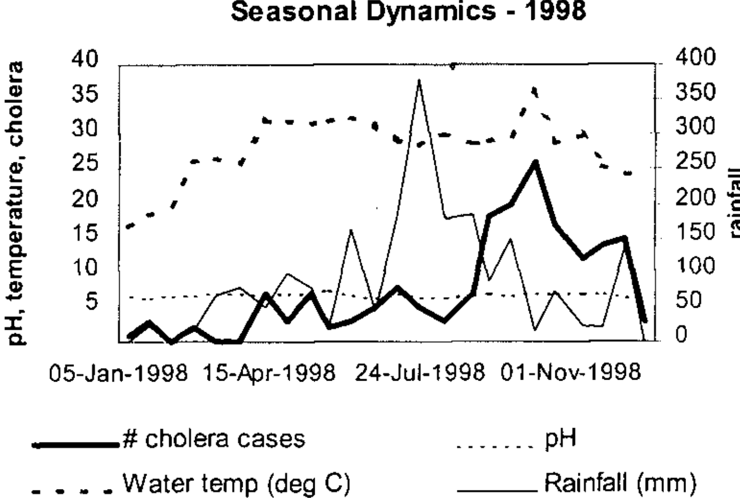


Fig. 4. Seasonal dynamics of pH and water temperature in a freshwater lake in Bangladesh, compared with rainfall and the number of cholera cases in the surrounding area. Note that that peak in cholera cases follows the peak in rainfall and coincides with a temperature peak.

Background of the Project including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the **significance and rationale** of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. **(DO NOT EXCEED 5 PAGES, USE CONTINUATION SHEETS).**

In pelagic ecosystems, heterotrophic bacteria are responsible for much of the remineralization of organic matter and as such can be key components of aquatic food webs and nutrient cycles (Schlesinger 1997). As a result, the conditions regulating bacterial abundance, distribution, and productivity can be vital to ecosystem function. Like all organisms, bacteria are phenotypically plastic and can change gene expression and consequently their phenotype in response to their environment. For example, bacteria can react to good or poor surrounding conditions by increasing or decreasing metabolic rates, including entering a distinct dormant state. Bacteria can also change the quality of their microhabitat by switching between free-living (unattached) and surface-attached forms. While attached, bacteria may exhibit group-level behavior called biofilm formation to increase their protection or nutrient acquisition from their surroundings.

Microarrays: Microarrays which measure whole-genome expression profiles are a powerful new tool for studying an organism's response to its environment (e.g., Hamadeh and Afshari 2000). A microarray consists of a glass microscope slide that contains up to several thousand cDNA or oligonucleotide spots that can be hybridized to differentially labeled products of reverse-transcribed bacterial RNA. Two factors facilitate our use of microarrays to look at *V. cholerae*: the availability of the complete genomic sequence for *V. cholerae* (Heidelberg *et al.* 2000) and a wealth of additional information resulting from extensive environmental, genetic and molecular studies on this species (reviewed by Huq and Colwell 1994). Thus, we propose to use microarrays and ecological analyses to correlate environmental parameters with the molecular events associated with bacterial behavior, especially attachment and dormancy. The ability to evaluate simultaneous expression of the entire genome allows for a detailed, mechanistic interpretation of an organism's reaction to its environment. Microarrays therefore provide information that cannot be obtained from the ecological techniques currently used to measure bacterial productivity, respiration, and enzyme production.

We will monitor bacterial response to environmental conditions by following the individual and aggregate expression of genes in specific categories, as well as the genome as a whole. Specifically, we will focus on (1) genes indicating varying levels of bacterial activity from fully dormant to rapid growth and cell division and (2) genes that regulate surface response behaviors (SRBs). SRBs are associated with genes that indicate preparation to change microhabitat (e.g., production of attachment or surface location factors by unattached bacteria, or detachment factors by attached bacteria), biofilm formation (if attached), and production of chitinase, mucinase, and other hydrolytic ectoenzymes specific to metabolizing surface material. We also expect to identify suites of previously uncharacterized genes that demonstrate significant differential expression with respect to microhabitat and water quality. A specific focus for this activity will be genes on the smaller chromosome, which are more poorly characterized than the genes located on the large chromosome (Heidelberg *et al.* 2000). Many of these genes have been hypothesized to play a critical role in environmental survival, since they have not been identified in the numerous studies dealing with virulence and general cell physiology (Heidelberg *et al.* 2000).

Productivity and Dormancy: Studies show that 30-95% of bacteria may be dormant in both aquatic (reviewed by Cole 1999) and terrestrial (Norton and Firestone 1991) ecosystems at any given time. The high dormancy rate suggests that (1) most bacterial production comes from a relatively small fraction of the population and (2) bacteria in most ecosystems have the potential to respond very rapidly to changes in environmental conditions that trigger the transition back to a metabolically active state (Cole 1999). This has important implications for nutrient cycling (Sterner *et al.* 1995), decomposition (Norton and Firestone 1991), and other ecosystem processes (Cole 1999). In the microbial literature, including *V. cholerae* studies, the dormant state is referred to as viable-but-nonculturable (VBNC). Interestingly, there is surprisingly little overlap between the epidemiological literature on pathogenic bacteria in the VBNC state (e.g., Huq *et al.* 2000) and the ecological literature on dormant bacteria in natural

ecosystems (e.g., Cole 1999). Although the large pelagic dormant population results partly from selective grazing of larger, metabolically active cells by nanoflagellates (Pace and Cole 1996, Lagenheder and Jurgens 2001), reduced productivity or even dormancy are also a response to sub-optimal environmental conditions (Cole 1999). Low temperature, N, P, and organic C, as well as extreme salinities and pH, are some of the factors which have been shown to affect bacterial physiology in aquatic systems. Importantly, the particular optimal values depend on the system to which the organism is adapted, and a variety of interactions between these factors are known (e.g., lake bacterial growth limited by temperature at low temperatures and by nutrients at high temperatures, Carlsson and Caron 2001).

Genes associated with productivity and dormancy: There are many indications, based on morphological (rods become small spheres: Baty *et al.* 2000, Huq *et al.* 2000) and physiological changes (e.g. decreased membrane fatty acids: Linder and Oliver 1989) that bacterial dormancy is a distinct condition, possibly similar to a resting stage like a *Daphnia* epiphium or an algal spore, rather than simply a lower state of metabolic activity. In spite of the ecological importance of bacterial dormancy, almost nothing is known about the molecular basis of transition into and out of this condition (but see Ravel *et al.* 1994, del Mar Lleo *et al.* 2000). Thus an exciting novel area for the application of microarrays is to begin to understand this process at the molecular level. We will monitor gene expression throughout the time courses of transitions into, and out of, bacterial dormancy. One suite of genes for which expression is expected to be altered coincident with progression into the dormant state are those that encode proteins involved in cell wall synthesis, corresponding to the change in cellular morphology that occurs during this process. In contrast, the transition from dormancy to productivity is likely to involve the activation of genes involved in a variety of metabolic processes. For attached organisms, these changes may be correlated with gene expression patterns suggesting mechanisms of release and transition into a free- swimming state. It is anticipated that numerous genes of unknown function will be coordinately regulated along with the genes of predicted function. These studies will provide the basis for future mutational analyses to dissect the important processes associated with bacterial dormancy.

Attachment: Macroscopic organic aggregates known as detrital aggregates, marine snow or lake snow are an important microhabitat for bacteria in pelagic systems because they provide locally abundant resources for attached cells. While attached individuals comprise a small percentage of the pelagic bacterial population, they may account for a large fraction of microbial productivity (Azam *et al.* 1994, Turley and Mackie 1994, Alldredge *et al.* 1986, Smith *et al.* 1995, Grossart and Simon 1998, Grossart and Pough 2000). However, production by bacteria associated with aggregates is not always greater than unattached bacteria (Mullerniklas *et al.* 1994, Turley and Mackie 1994, Middleboe *et al.* 1995, Unanue *et al.* 1998, Turley and Stutt 2000). For example, the relative productivity of attached and unattached bacteria depends on such factors as seasonal water conditions, and type and age of attachment substrate (Grossart and Pough 2000). Studies with microbial media have found that nutrient availability and water chemistry mediate surface colonization rates (Davey and O'Toole 2000, O'Toole *et al.* 2000, hiavelli *et al.* submitted: Fig. 1). It is difficult to know how well these studies apply to natural conditions, but presumably changes in natural water conditions such as nutrient levels or salinity may also affect the tendency to attach. Several studies indicate that poor environmental conditions or more nutritive surfaces enhance bacterial attachment in natural conditions. Baty *et al.* (2000) found greater colonization on chitin than on a non-nutritive substrate. Huq *et al.* (1984b) found that colonization of copepods by *V. cholerae* increased as salinity increased from 5 to 15 parts per thousand. Furthermore, bacterial cells starved to mimic the small inactive morphology common in oligotrophic aquatic systems will readily attach to substrates (Power and Marshall 1988, Baty *et al.* 2000). We will examine how both water and substrate conditions mediate gene expression of attachment factors in natural conditions.

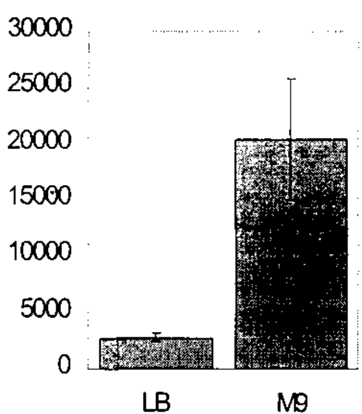


Fig. 1. Number of attached cells per mm^2 on *Daphnia* exoskeletons after a 2-hr attachment assay with *V. cholerae* O1 El Tor at ~10^6/ml in nutrient rich (LB) and nutrient limited (M9) media. From Chiavelli *et al.* (submitted).

Given that attached bacteria are common on many zooplankton and phytoplankton (Nagasawa 1989, Threlkeld *et al.* 1993, Jensen *et al.* 1996, Carman and Dobbs 1997; Islam *et al.* 1994a; Fig. 2), it is surprising that research on the ecosystem-level impacts of attached pelagic bacteria ignores zooplankton and almost completely ignores phytoplankton as

surface microhabitats (phytoplankton can inhabit detrital aggregates so are sometimes considered in that perspective). Conversely, *V. cholerae* researchers often find this bacterium on zooplankton and phytoplankton, but have not considered attachment to detrital particles. This discrepancy is perhaps a result of a traditional ecological focus on bacteria as decomposers vs. a focus on host-parasite scenarios in microbiology. In addition to surface-associated nutrients, an advantage of attachment to plankton as compared to detrital particles is access to excreted nutrients. Clearly, plankton surfaces provide an enormous amount of pelagic bacterial microhabitat and this work provide new information about the potential significance of attachment to plankton for bacterial population dynamics and ecosystem function.

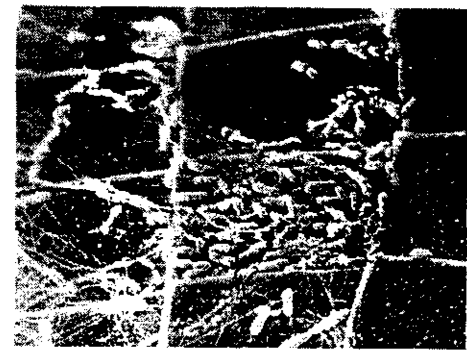


Fig.2. SEM photograph of bacteria attached to *Daphnia*.

Detachment: Although detachment is undoubtedly a vital process in bacterial population dynamics and bacterially-mediated ecosystem functions, detachment from planktonic substrates has not been studied in natural pelagic ecosystems. In fact, virtually nothing is known about bacterial detachment in any system (O'Toole *et al.* 2000). Detachment probably correlates with substrate age due to resource depletion (Grossart and Pough 2000, Baty *et al.* 2000, O'Toole *et al.* 2000). For example, Baty *et al.* (2000) found that starved cells of a marine bacterium readily colonized chitin and subsequently up-regulated genes related to chitinase (*chiA-chiB*). After attached numbers stabilized, *chiA-chiB* decreased in expression, and detachment rates increased. Detached cells had little or no expression of *chiA-chiB* genes. This pioneering study indicates that bacterial movement among microhabitats in pelagic ecosystems could be mediated both by nutritional state and local resource availability. Our work will provide additional information about bacterial detachment both on a molecular level and ecologically in terms of the role of water conditions, substrate type, and substrate age in inducing bacterial detachment.

Biofilms: Following attachment, organization of attached bacteria varies in complexity from unassociated cells to well-organized, three-dimensional structures known as biofilms adapted to take advantage of interface conditions to increase their productivity (Costerton *et al.* 1995, O'Toole & Kolter 1998, Davey and O'Toole 2000, O'Toole *et al.* 2000). The formation of a mature biofilm involves aggregation of bacteria followed by secretion of exopolysaccharides and colony development. Colonies are interspersed with water channels that allow efficient transport of nutrients (O'Toole & Kolter 1998, Davey and O'Toole 2000, O'Toole *et al.* 2000). Like the ecologists who study attachment to detrital particles, microbiologists currently believe that the majority of bacterial production in natural systems is the result of biofilms rather than unattached bacteria (Costerton *et al.* 1995, Davey and O'Toole 2000, O'Toole *et al.* 2000). Bacteria biofilm activity on detrital or living substrates is probably common, but is virtually unstudied for pelagic aquatic ecosystems (Cooksey and Wigglesworth-Cooksey 1995).

Genes associated with attachment, detachment, and biofilm formation: V. cholerae is one of the best-studied model organisms for the genetic regulation of SRBs. Genetic factors regulating transition from the unattached to the attached condition include production of pili, outer membrane proteins, degree of motility, and exopolysaccharides (O'Toole and Kolter 1998, Watnick and Kolter 1999, Davey and O'Toole 2000, O'Toole *et al.* 2000, Yildiz *et al.* 2001, Chiavelli *et al.* submitted: Fig. 3). Biofilm formation and detachment are influenced by broad genetic regulatory systems such as quorum sensing, carbon utilization and stationary phase regulons. Genomic studies will allow us to address a number of ecologically relevant questions at the level of gene expression. For example, what environmental conditions cause unattached bacteria to be 'looking for' a surface, as evidenced by genes encoding surface detection and attachment factors? How active are bacteria on that surface, as evidenced by genes regulating biofilm development and uptake of surface associated nutrients? When do bacteria detach from a surface, as indicated by increased expression of genes encoding flagellar proteins, polysaccharide cleaving lyases, and other detachment-associated enzymes? In this context, our assessment of genes which regulate SRBs of unattached bacteria and bacteria on two types of planktonic surfaces in a wide range of environmental conditions will be an enormous and novel contribution to knowledge of the role of bacteria in aquatic ecosystems.

Study Organism: Vibrio cholerae is an excellent model organism for studying bacteria in pelagic systems. It is a globally distributed bacterium found in marine, estuarine and freshwater locations (e.g., Colwell *et al.* 1981, Huq *et al.* 1983, West and Lee 1982, Garay *et al.* 1985, Nair *et al.* 1988, Dumontet *et al.* 1996, Carvajal *et al.* 1998, Falcão *et al.* 1998, Lowenhaupt *et al.* 1998, Islam *et al.* 1994a). It is a member of the *Vibrionaceae*, a predominant group of heterotrophic bacteria with numerous aquatic representatives. Like other members of its family, *V. cholerae* can exist in either the attached or unattached state and is often found attached to phytoplankton or crustacean

zooplankton (Huq *et al.* 1983, 1984a,b, 1990, 1996; Islam *et al.* 1988, 1989, 1990, 1993, 1994, 1996, 1999; Tamplin *et al.* 1990, Dumontet *et al.* 1996, Chowdhury *et al.* 1997, Hood and Winter 1997). Like many other vibrios, *V. cholerae* can produce both chitinase and mucinase (Dastidir and Naranyanaswami 1968, Nalin 1976, Nalin 1979, Schneider and Parker 1982), indicating it also has the ability to directly metabolize the surface material of crustacean zooplankton and mucilage-sheathed phytoplankton. Due to its medical importance, its genome is fully sequenced (Heidelberg *et al.* 2000) and well studied, allowing us to take advantage of microarray technology to study its response to changing environmental conditions.

There is a great deal of information about the interplay of SRBs and water quality from laboratory experiments involving colonization of and growth on planktonic substrates, as well as field studies of attachment to and viability on plankton, particularly for *V. cholerae*. *V. cholerae* survival increases when the bacterium is associated with zooplankton and phytoplankton (Huq *et al.* 1984a,b, Islam *et al.* 1988, 1989, 1990, 1999). Several studies have evaluated attachment of *V. cholerae* to plankton substrates under different environmental conditions (pH, salinity, temperature, nutrients; Huq *et al.* 1984a, McCarthy 1996, Hood and Winter 1997, Chiavelli *et al.* submitted), although none have addressed interactions between these factors. *V. cholerae* attached to zooplankton in the field are usually found to be dormant (Huq *et al.* 1990, 2000), in direct contrast to the usual finding of higher productivity for bacteria on detrital particles than for unattached bacteria. A possible reason for this discrepancy is substrate age, especially for long-lived zooplankton species, which could lead to local depletion of resources and dormancy even if metabolism is briefly elevated after attachment to plankton. Additionally, *V. cholerae* may attach to zooplankton and enter dormancy as a strategy to persist through times of the year with poor conditions. Thus attachment to zooplankton could serve either as productive location for population growth or as protective location for dormant bacteria. One of the contributions of the proposed work will be to help resolve this contradiction.

In contrast to zooplankton, *V. cholerae* that have colonized the mucilaginous sheaths of *Anabaena* colonies remain metabolically active (dividing cells observed) for long periods in laboratory studies, probably as a result of organic carbon and fixed nitrogen produced by this substrate organism (Islam *et al.* 1999). We will determine how these two very different substrates, zooplankton exoskeletons and colonial cyanobacteria, affect expression of genes that regulate dormancy and SRBs.

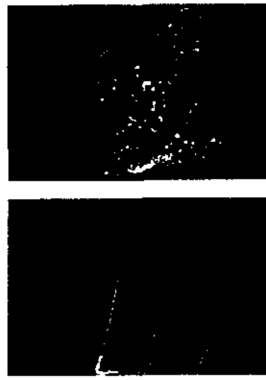


Fig.3. *Daphnia* exoskeletons after a 2-hr attachment assay with *V. cholerae* O139 (expressing green fluorescent protein) at ~10^6/ml. Top exposed to *mshA*$^+$ strain (wild type), bottom to MSHA pilus-deficient mutant. Bright dots are bacteria, no mutants were able to attach.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children. use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project. **(DO NOT EXCEED TEN PAGES, USE CONTINUATION SHEETS).**

Methods for hypothesis 1:

Preliminary Experiments: Since we are particularly interested in the transition into dormancy, we will run preliminary experiments to determine the effects of different nutrient concentrations on bacterial activity. Activity will be assessed using cell morphology and the percent of dormant cells as determined by the fluorescent antibody-direct viable count (FA-DVC) method routinely used by the Huq lab and at the ICDDR,B (Chowdhury *et al.* 1995). We will start the pilot experiments with actively growing cells from a log-phase culture, then identify (1) a low nutrient level which induces fairly rapid transition into dormancy (<2 d) and a high nutrient level for which a large percentage of the cells remain culturable for our 14 d experiments. All nutrient treatments will be conducted at the Redfield C:N:P ratio, with inorganic N and P manipulated using NH_4Cl and NaH_2PO_4 and organic C manipulated using glucose. The nutrient levels will be the same as used. We expect to use nutrient concentrations comparable to the minimum and maximum levels in the ponds in Bangladesh where our *V. cholerae* strain was isolated.

Experimental Design: Genomic response of *V. cholerae* will be quantified at least daily (0, 6, 12, 24 hrs then every 24 hr) over 14 d to allow us to observe phenotypic plasticity in dormancy or attachment. For example, we would like to observe unattached bacteria entering and emerging from dormancy, and potential changes in behavior of attached bacteria as easily metabolized substances are depleted, such as switching to more refractory substances, detachment, or entering a dormant state. As described above, there will be six experiments, run over a period of ~1.5 years: two initial conditions (log-phase or dormant cultures) crossed with three bacterial microhabitats (bacteria grown without any plankton substrates for attachment, bacteria allowed to colonize exoskeletons of a crustacean zooplankton and subsequently sampled, and bacteria allowed to colonize *Anabaena* colonies and subsequently sampled. Within each experiment, we plan to have three independent replicates of two low and high nutrient treatments as determined by our preliminary experiments (above), for a total of 2 treatments x 3 replicates x 17 times = 102 microcosms. All experiments will be conducted at 30°C, a salinity of 15‰, and pH 7. At each sampling time bacteria will be processed for microarray analysis and the determination of the percent of dormant cells (Chowdhury *et al.* 1995). The independent replicates allow for destructive sampling, which eliminates statistical problems of repeated observations from the same experimental unit (Underwood 1997) and difficulties in subsampling without affecting experimental conditions.

Experimental Procedure: Experiments will be conducted with *Vibrio cholerae* O1 El Tor strain number RC2592 isolated from Bangladesh. This strain has been chosen because it is a non-pathogenic, environmental isolate that matches the available genomic sequence of El Tor clinical strain N16961. Prior to experiments bacteria will be (1) be grown in an overnight culture started from frozen samples for the metabolically active initial treatment or (2) maintained in filter-sterilized artificial sea water (Instant Ocean) modified to 15‰ salinity to induce dormancy as described in Chayianan et. al. (submitted). Experiments will take place in filter-sterilized pond water. *V. cholerae* will be inoculated at 10^6/ml along with planktonic substrates (if relevant) into filter-sterilized pond water modified to represent the intended experimental treatment conditions, then time zero samples will be taken to determine initial gene expression. Experimental containers will be clear PVC tubes, 50cm high, ~5cm diameter, plugged at both ends. These ~1L containers will be overturned 4 times per day during the experiments in order to keep materials mixed and especially to allow the planktonic surfaces to be continuously sinking. This is necessary as continuous flow past surfaces is important in maintaining field-relevant productivity levels (Grossart and Plough 2000). Incubators will kept at a light level representative of 0.25 m depth in a typical eutrophic pond.

Plankton Substrates

- *Daphnia: V. cholerae* attach to both live zooplankton (*Daphnia* sp., other cladocera, cyclopoid and calanoid copepods, rotifers) and to zooplankton exoskeletons in Bangladesh ponds (Huq *et al.* 1990, Tamplin *et al.* 1990). *V. cholerae* are usually more abundant on copepods than on cladocera and so are thought to be a preferred host, but this difference may be because cladocera molt every few days throughout their lives while copepods do not molt as adults. Bacteria are shed when zooplankton molt, so that burden of attached organisms increases with time since molting (Threlkeld *et al.* 1993, Willey and Threlkeld 1995, Al-Daheri and Willey 1996). We have found that *V. cholerae* will readily colonize *Daphnia* exoskeletons in laboratory experiments (Chiavelli *et al.* submitted), plus *Daphnia* have logistical advantages because they are easier to keep alive in culture and molt frequently. To avoid complications of including live zooplankton in our experiments, we will use freshly shed (<24hr old) *Daphnia* exoskeletons that have been pre-sonicated and rinsed to remove bacteria.
- *Anabaena*: We will use *Anabaena variabilis*, a nitrogen-fixing, mucilage-sheathed cyanobacterium, from a standard axenic laboratory culture as our phytoplankton substrate. We chose *Anabaena* as our focal species because it is extremely abundant in Bangladesh (Islam *et al.* 1994) and many other eutrophic systems (Reynolds 1984), has a mucilage sheath known harbor *V. cholerae* (Islam *et al.* 1989, 1990, 1994), and increases survival of *V. cholerae* in laboratory studies (Islam 1990, 1999).

Sample processing: After rinsing to remove unattached bacteria, attached bacteria will be removed and concentrated by alternating vortex mixing and sonication using an ultrasonic cleaner according to the method of McSpadden Gardner and De Bruijn (1998). As a control, the same procedure will be used in the experiments with unattached bacteria. Exoskeletons can be removed with tweezers, but separation of *V. cholerae* from *Anabaena* may present a technical challenge. One strategy will be to incubate the bacteria with anti-O1 *V. cholerae* LPS antibodies and then separating the *V. cholerae* from the *Anabaena* by using magnetic bead coupled secondary antibodies and benchtop magnetic separation (Pierce, Inc.). Contamination of the sample with *Anabaena*, a cyanobacterium, only represents a problem if standard random priming is used to detect RNA expression because of potential cross hybridizing *Anabaena* and *Vibrio* sequences. An alternative procedure will be to use a *V. cholerae* gene-specific primer set for the reverse transcriptase step. In this case, a mixture of 3' primers that represent each gene on the array will be used at molar excess, rather than a random primer mix, for the reverse transcriptase reaction (see below in microarray design).

Microarray Design: We have chosen to use glass microarrays, rather than lithographic DNA chips, due to considerations of cost and flexibility. Cost prohibits the use of lithographic chips unless they are commercially available for the organism of interest or there is a large consortium of investigators who can share the cost. Neither of these exist in the case of *V. cholerae*, or for the many microbes for which environmental studies will be undertaken. In addition, we envision that for some of our future studies, we will be able to customize our arrays to harbor only the genes of interest. Such arrays can be customized in house at greatly reduced cost.

The Taylor laboratory has carried out its initial microarray analysis on 2500 human genes using arrays available from NEN to study changes in host gene expression that accompany colonization by *V. cholerae*. The *V. cholerae* microarrays are currently being developed with fiscal and technical support from other grants to the Taylor lab and institutional support for genomic analysis. Thus we are familiar with all the technical manipulations and methods of data analysis required for a large-scale project and the proposed studies benefit greatly from prior development funded from other sources. Our *V. cholerae* microarray represents all of the predicted protein-encoding genes that have been characterized by the sequencing project carried out by TIGR. This includes 2775 ORFs on the large chromosome and 1115 on the small chromosome of El Tor biotype strain N16961. We have chosen an El Tor environmental isolate for the experiments in this proposal. To help normalize our data, we have also included genes that are predicted to vary only slightly with growth conditions, such as aminoacyl tRNA synthetase genes that have been shown to vary less than 2.5 fold even under the extremely different growth conditions of minimal versus rich media (Tao *et al.* 1999). In addition, total pooled RNA standards are spotted onto each microarray to serve as a fluorescence intensity reference (Wei *et al.* 2001; K. Guillemin, personal communication). The targets are PCR products that are generally of 300-400 bp in length. Approximately 10 ng of target is spotted in a 7 nl volume onto gamma amino propyl silane coated microscope slides (Corning, Inc.). These slides are then treated according to the conditions suggested by Eisen and Brown (1999) with slight modifications. The probe preparation for analysis of gene expression follows the methods of Richmond *et al.* (1999) with slight modifications provided by Craig Richmond. RNA is isolated by the hot phenol method as we have performed in the past (Brown and Taylor 1995). After DNaseI treatment, it is further purified using a RNeasy column (Qiagen). For the time course experiments

described in this aim, we will chose one set of experiments where RA isolated immediately at each time point and a parallel sample is saved for subsequent analysis using RNAlater (Pierce, Inc.) which stabilizes RNA in bacterial samples. The results using these parallel samples will be compared in order to evaluate whether RNAlater can be applied to facilitate the experimental plan for Aim 3. In all cases, the quality of the RNA preparation is critical for this analysis and will be monitored by visualizing the rRNA pattern after gel electrophoresis of the preparations. 20ug of RNA is reverse transcribed in a reaction that is primed by random hexamers or the species specific 3' primer set described in the previous section. The reactions contain Cy3 or CyDye-dUTP (Amersham Pharmacia Biotech) in a dNTP mix that has a decreased amount of dTTP. The RNA is subsequently degraded in the presence of NaOH and the cDNA is purified on a microcon 30 concentrator. Hybridization to the arrays is performed under standard conditions in a humidity controlled hybridization chamber. The slides are then washed, spun dry on microtiter plate carriers and scanned using a GMS 418 scanner and Jaguar® software.

Methods for hypothesis 2:

Experimental Design: For each of the three microhabitats we will conduct a completely-crossed, 2^4, center-point-enhanced factorial experiment: four environmental factors at two treatment levels each, all possible combinations examined, with four additional center point treatments where all four factors are midway between the high and low levels used in the other treatments (Table 2). One replication of our experiment will therefore require 20 experimental units (culture beakers and microarray chips) and will provide eight replicates of the direct effects of each environmental factor, four replicates of each two-factor interaction, and two replicates of each 3-factor interaction. The 4-factor interaction can also be resolved because the center points provide an independent estimate of variance (Montgomery 1991). Center point treatments in a design also give a general estimate of non-linearity (as "lack of fit" at the center point) while using very few additional experimental units (Montgomery 1991). In addition to being a very efficient design, the 2^k factorial is also more scientifically robust than several one-factor experiments, because it tests for interactions among factors that can be critical to explaining system behavior. Each experiment will use microarray chips from a single batch production and all treatments will proceed simultaneously to reduce technical and methodological variation. All chips will also receive RNA from a single reference which we will use to correct for variance among chips.

Once one experiment from each microhabitat is analyzed, we will take one of two directions. First, if the variance due to error is too large for us to statistically detect effect sizes of a three-fold change in gene expression, we will repeat the experiment and consider the two experiments replicates blocked by time. This additional replication will give us more power to separate biological effects from background variance. Alternatively, if random variance among chips is low and factors and interactions with strong biological effects have been identified, we will design a second experiment with fewer factors and more levels per factor. RNA used from previous experiments will be saved and applied to chips used in these experiments in order to calibrate responses across multiple sets of experiments.

Procedure:

Treatment Factors: As stated in the overview, the environmental treatment factors have been chosen because of their ecological and physiological relevance, and the levels of the factors will span the seasonal range of values in the Bangladesh ponds and estuaries where this strain occurs (Oppenheimer *et al.* 1978, A. Huq and B. Sack unpublished data).

- *Nutrients:* We will compare low versus high nutrient availability using the same levels as in Aim 1. Aquatic bacterial growth in the field is generally limited by one or more of these three nutrients (e.g. Pace and Cole 1996, Carlsson and Caron 2001). *V. cholerae* growth and survival increase with the concentration of organic nutrients in water (Singleton 1982), and higher colonization rates of *V. cholerae* have been found in nutrient poor water (Huq *et al.* 1996, Islam *et al.* 1999) and nutrient limiting medium (Chiavelli *et al.* in preparation; Fig. 1).
- *Temperature*: We will compare 20°C and 30 °C, roughly the cold and warm season water temperatures in Bangladesh ponds (Oppenheimer *et al.* 1978; Fig. 4). Low temperatures can limit bacteria growth and survival in aquatic systems (e.g., Carlsson and Caron 2001), particularly of tropical species like *V. cholerae* (Xu *et al.* 1982, Huq *et al.* 1984b). Huq *et al.* (1984b) and Hood and Winter (1997) have found that colonization of zooplankton increases with temperature, but these results may have been confounded by the faster growth rate in higher temperatures.
- *Salinity:* We will compare salinities of 0 (freshwater) and 15‰ (estuarine). Because *V. cholerae* is found both in the freshwater ponds of our field study and nearby estuarine systems, we are interested in comparing bacterial gene expression in these contrasting salinities because their different physiological demands may change the way the bacteria respond to other conditions. *V cholerae* growth is optimal at intermediate (estuarine) salinity with freshwaters representing a fairly harsh habitat unless concentrations of other ions, including nutrients, are high (Hood and Winter 1997).
- *pH*: We will compare pH 7 (the mean pH in Bangladesh ponds, Fig. 4) and pH 8.5 (the typical afternoon pH during algal blooms, Oppenheimer *et al.* 1978) using HCl or NaOH to adjust pH. *V. cholerae* grow better at slightly basic than in neutral or acidic conditions (Huq *et al.* 1984b), and have been shown to remain in the culturable state longer in higher pH (Islam *et al.* 1990, 1999). One study finds that *V. cholerae* colonization increases with pH (Huq *et al.* 1984b), while another finds the opposite (Hood and Winter 1997). However, neither study separated colonization and growth on the plankton surface.

Table 2. Aim 2 experimental design. T: temperature, N: nutrients, S: salinity, P: pH. 1: high, 0: low level for each factor. The last four runs are center points: intermediate level of every factor. Letters in the treatment combination column indicate which factors will be at the high level in each run.

Run	Factor				Treatment
Number	T	N	S	P	Combination
1	0	0	0	0	0
2	1	0	0	0	T
3	0	1	0	0	N
4	1	1	0	0	TN
5	0	0	1	0	S
6	1	0	1	0	TS
7	0	1	1	0	NS
8	1	1	1	0	TNS
9	0	0	0	1	P
10	1	0	0	1	TP
11	0	1	0	1	NP
12	1	1	0	1	TNP
13	0	0	1	1	SP
14	1	0	1	1	TSP
15	0	1	1	1	NSP
16	1	1	1	1	TNSP
17	0.5	0.5	0.5	0.5	0.5(TNSP)
18	0.5	0.5	0.5	0.5	0.5(TNSP)
19	0.5	0.5	0.5	0.5	0.5(TNSP)
20	0.5	0.5	0.5	0.5	0.5(TNSP)

Methods for hypothesis 3:

Experimental Design: Our field experiments will evaluate bacterial gene expression in *in situ* incubation containers following a nested design where microhabitats are nested within enclosures, within seasons, within years, and within lakes. We will work in two ponds in Dhaka, Bangladesh, one in the Matlab agricultural area and one in Chandpur, near Matlab, a religious retreat relatively protected from sewage and agricultural runoff. We will perform experiments will be six times per year (timed as described above) over 2.5-3 yr to allow for interannual comparisons. During each experiment, we will use four enclosures per pond to account for spatial heterogeneity and sample our three microhabitats (unattached, on exoskeletons, and on *Anabaena*) in each enclosure.

Procedure: A non-toxigenic strain of O1 El Tor *V. cholerae*, along with *Daphnia* exoskeletons and *Anabaena*, our two substrates from Aims 1 and 2, will be placed in light-penetrable 1L enclosures with dialysis membranes to allow continuous equilibration with nutrients and other chemicals outside the enclosures. Enclosures will be filled with filter-sterilized water collected at the depth (0.25 m) where the incubation will occur. RNA will be processed as in Aim 1 at the ICDDR,B, and returned to Dartmouth for microarray processing. RNA samples will be harvested three days after the containers are set up to allow time for *V. cholerae* to colonize substrates and adjust to field conditions.

In order to thoroughly correlate our results with environmental trends, we will obtain biweekly measurements of temperature, dissolved oxygen, pH, conductivity, salinity, nitrate, nitrite, orthophosphate, total phosphate, chlorophyll, dissolved organic carbon, iron, and total dissolved solids in eachpond. In conjunction with each experiment, we will collect and preserve phytoplankton and zooplankton samples for identification and enumeration, and measure the percent of dormant bacteria in all three microhabitats in our experiments using methods described in Aim 1.

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out the laboratory facilities and major equipments that will be required for the study. For field studies, describe the field area including its size, population, and means of communications. (TYPE WITHIN THE PROVIDED SPACE).

This project will receive infrastructure support for development and outreach efforts from the new Center for Environmental Health at Dartmouth (DEHS, Josh Hamilton, Director). This Center seeks to foster collaborative projects that combine ecological research with human health and environmental quality issues. Support from the Outreach Core and Training Core programs will assist with the development of courses for graduate students and creation of undergraduate research opportunities.

R. Taylor has played a major role in developing the Molecular Genomics Core. Soon after the annotated sequence of the *V. cholerae* genome became available in late fall 1999, Taylor prepared an in-house proposal for the purchase of microarray hardware and software. The proposal was funded mainly from the Dean of the Medical School and from the new Department of Genetics. These funds were used to purchase a Genetic Microsystems 417 arrayer, a 418 scanner, and related software. We work together with, and partially fund, C. Ringelberg who is the lead technical person of the Molecular Genomics Core facility. We plan to work with DEHS, the Center for Biological and Biomedical Computing, and the Biostatistics Core on the development of programs for analysis of microarray data.

Dartmouth is committed to building on its strong foundation of computing and internet communication in combination with its new genetics initiative to achieve a leading role in genome-wide expression analysis and the dissemination of related information. For example, Dartmouth has a consortium of researchers working together to develop tools for genomic analysis that can be used through our web site (described below). The Microarray Research Group at Dartmouth meets regularly and consists of researchers from about 15 different labs including representatives that head up several international genomic based projects. We are setting up a comprehensive microarray database along with integrated analytic tools that will allow users to store and analyze their data through a web browser interface. The system will be based on one currently running at Stanford (http://genome-www4.stanford.edu/MicroArray /SMD/), and we are collaborating with Stanford to port the site to Dartmouth. This will facilitate our ability to share *V. cholerae* gene expression profiles with the research community at large. We will also make our arrays available "at cost" to other academic laboratories upon request.

Our proposed work also has a strong international component, with field work in Aim 3 to be directed by Dr. Sirajul Islam and technical personnel at the ICDDR in Dhaka, Bangladesh.

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical softwares packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. **(TYPE WITHIN THE PROVIDED SPACE).**

Because microarrays capture data on the simultaneous behavior of a large number of genes, analyzing results from microarray responses to a multifactor experiment requires a multivariate analysis of both the independent (experimental factors) and dependent variables (genes). We believe that the best approach to analyzing data from our experiments is to adopt a procedure developed by experimental community ecologists for analyzing the responses of multiple species to experimental treatments (e.g., Seabloom *et al.* 1998). This approach is conceptually similar to that developed by Alter *et al.* (2000) for single-factor experiments.

The community ecology approach has two steps. First, principal components analysis (PCA) is used to extract orthogonal axes called principal components (or PC's) that explain significant amounts of the variability in responses among genes; these axes are linear functions of genes and can therefore be related back to the microarray data. Second, analysis of variance (ANOVA) is applied to each PC that explains more than some threshold fraction of the overall variation (say 10%) to quantify the effects of the experimental treatments. For Aim 1, we will use three-way ANOVA to quantify the effects of microhabitat, treatment (low/high nutrients), and time, as well as each two- and three-way interaction. Aims 2 and 3 will use ANOVA models appropriate to their design. All analyses will be conducted using SAS Statistical Software.

We will use this two-step procedure for two distinctly different analyses of the microarray data. First, we will conduct focused analyses of groups of genes with a priori associations with metabolic activity (growth, productivity), dormancy, and SRBs in order to test specific hypotheses about the effects of environmental conditions on particular bacterial activities. Second, we will conduct a composite analysis of the entire genome in order to detect patterns in other suites of genes, including those whose function is not yet known. Certain patterns of gene expression can be predicted. For example, we hypothesize that genes controlled by the stationary phase RNA polymerase sigma factor RpoS will be among those induced by starvation and transition into dormancy. This is a well documented genetic regulon in many bacteria that is of the type we will use as a standard for correlation of activation for additional genes that are induced under these conditions. Other such regulons respond to nitrogen and phosphate.

We will carefully evaluate the PCA + ANOVA approach using data from our pilot experiments, and will modify this approach as needed. In addition, new techniques for dealing with multivariate responses to experimental treatments using Mantel tests are currently in development (Legendre and Legendre 1998; E. Seabloom, pers. comm), and we expect to try them out on microarray data as they become established in the literature.

As in Aim 1, we will analyze the results of this experiment using a combination of PCA and ANOVA as applied to specific gene groups as well as the entire genome. The ANOVA component of this analysis will follow the procedures described by Montgomery (1991) for a 2^4 center-point enhanced factorial experiment, with the addition of a fifth factor for microhabitat.

As in Aims 1 and 2, we will analyze the result of the field experiments using a combination of PCA and ANOVA applied to specific gene groups as well as the entire genome. For Aim 3, the experimental design involves a hierarchical nested design: lake, year, season, enclosure, and microhabitat. This design is straightforward to analyze in SAS.

In addition to these multivariate analyses, if the laboratory experiments have identified particular indicator genes for environmental response, we will apply ANOVA to these individual genes of special interest as well as to the groups of genes.

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

Not Applicable

Use of Animals

Describe in the space provided the type and species of animal that will be used in the study. Justify with reasons the use of particular animal species in the experiment and the compliance of the animal ethical guidelines for conducting the proposed procedures.

Not Applicable

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

Al-Dhaheri, R. S., and R. L. Willey. 1996. Colonization and reproduction of the Epibiotic Glagellate *Colacium vesiculosum* (Euglenophyceae) on *Daphnia pulex*. J. Phycol. **32**:770-774.

Alldredge,A. L, J. J. Cole and D. A. Caron. 1986. Production of heterotrophic bacteria inhabiting macroscopic organic aggregates (marine snow) from surface waters. Limnol. Oceanogr. **31**:68-78.

Alter, O., P. O. Brown, and D. Botstein. 2000. Singular value decomposition for genome-wide expression data processing and modeling. Proc. Natl. Acad. Sci. USA **97**:10101-10106.

Azam, F., D. C. Smith, G. F. Steward, and A. Hagstrom. 1994. Bacteria-organic-matter coupling and its significance for oceanic carbon cycling. Mirobial Ecology **28**:167-179.

Baty, A. M., C. C. Eastburn, S. Techkarnjanaruk, A. E. Goodman, and G. G. Geesey. 2000. Spatial and temporal variations in chitinolytic gene expression and bacterial biomass production during chitin degradation. Appl. Environ. Microbiol. **66**:3574-3585.

Brown, R.C. and R.K. Taylor. 1995. Organization of *tcp*, *acf*, and *toxT* genes with a ToxT-dependent operon. Mol. Microbiol. **16**:425-439.

Carlsson, and A. Caron. 2001. Seasonal variation of phosphorus limitation of bacterial growth in a small lake. Oceanogr. **46**:108-120.

Carvajal, G. H., J. Sanchez, M. E. Ayala, and A. Hase. 1998. Differences among marine and hospital strains of *vibrio cholerae* during Peruvian epidemic. J. Gen. Appl. Microbiol. **44**:27-33.

Chaiyanan, S., A. Huq, T. Maugel, and R.R. Colwell. Viability of the coccoid form of *V. cholerae* O1 and O139. J. Syst. Appl. Microbiol. Submitted.

Chiavelli, D.A., J.W. Marsh, and R.K. Taylor. The mannose-sensitive hemagglutinin of *Vibrio cholerae* promotes adherence to zooplankton. Appl. Environ. Microbiol. Submitted.

Chowdhury, M. A. R., A. Huq, B. Xu, F. J. B. Madeira, and R. R. Colwell. 1997. Effect of alum on free-living and copepod-associated *Vibrio cholerae* O1 and O139. Appl. Environ. Microbiol. **63**:3323-3326.

Chowdhury, M. A. R., B. Xu, R. Montilla, J. A. K. Hasan, A. Huq, and R. R. Colwell. 1995. A simplified immunofluorescence technique for detection of viable cells of *Vibrio cholerae* O1 and O139. J. Microbiol. Methods **24**:165-170.

Cole, J. J. 1999. Aquatic microbiology for ecosystem scientists: new and recycled paradigms in ecological microbiology. Ecosystems **2**:215-225.

Colwell, R.R. and A. Huq. 1994. Vibrios in the environment: viable but nonculturable *Vibrio cholerae*. P. 117-133 *in* I.K. Wachsmuth, P.A. Blake, and O. Olsvik. *Vibrio cholerae* and Cholera: Molecular to Global Perspectives. American Society for Microbiology, Washington, D.C.

Colwell, R. R., R. J. Seidler, J. Kaper, S. W. Joseph, S. Garges, H. Lockman, D. Maneval, H. Bradford, N. Roberts, E. Remmers, I. Huq, and A. Huq. 1981. Occurrence of *Vibrio cholerae* serotype O1 in Maryland and Louisiana estuaries. Applied and Environmental Microbiology **41**:555-558.

Cooksey, K. E., and B. Wigglesworth-Cooksey. 1995. Adhesion of bacteria and diatoms to surfaces in the sea - a review. Aquat. Microb. Ecol. **9**:87-96.

Costerson, J. W. , Z. Lewandowski, D. E. Caldwell, D. R. Korber, and H. M. Lappin-Scott. 1995. Microbial biofilms. Ann. Rev. Microbiol. **49**:711-745.

Dastidir, S. G., and A. Narayanaswami. 1968. The occurrence of chitinase in vibrios. Indian J. Med. Res. **56**:654-659.

Davey, M. E., and G. A. O'Toole. 2000. Microbial biofilms: from ecology to molecular genetics. Microbiol. Mol. Biol. Rev. **64**:847-867.

Del Giorgio, P. A. and J. J. Cole. 1998. Bacterial growth efficiency in natural aquatic systems. Ann. Rev. Ecol. Syst. **29**:503-541.

del Mar Lleo M, Pierobon S, Tafi MC, Signoretto C, Canepari P. 2000. mRNA detection by reverse transcription-PCR for monitoring viability over time in an *Enterococcus faecalis* viable but nonculturable population maintained in a laboratory microcosm. Appl Environ Microbiol. **66**:4564-4567.

Dumontet, S., K. Krovacek, S. B. Baloda, R. Grottoli, V. Pasquale, and S. Vanucci. 1996. Ecological relationship between *Aeromonas* and *Vibrio* spp and planktonic copepods in the coastal marine environment in southern Italy. Comp. Immunol. Microbiol. Infect. Dis. **19**:245-254.

Eisen, M.B. and P.O. Brown. 1999. DNA arrays for analysis of gene expression. Methods of Enzymol. 303:179-205.

Falcao, D. P., W. R. Lustri, and T. M. Bauab. 1998. Incidence of non-01 *Vibrio cholerae* and *Aeromonas* spp. in fresh water in Araraquara, Brazil. Current Microbiol. **37**:28-31.

Garay, E., A. Arnau, and C. Amaro. 1985. Incidence of *Vibrio cholerae* and related vibrios in a coastal lagoon and seawater influenced by lake discharges along an annual cycle. Appl. Environ. Microbiol. **50**:426-430.

Glass, R. I., S. Becker, M. I. Huq, B. J. Stoll, M. U. Khan, M. H. Merson, J. V. Lee, and R. E. Black. 1982. Endemic cholera in rural Bangladesh, 1966-1980. Amer. Journ. Epidemiol. 116:959-970.

Grossart, H. P., and H. Ploug. 2000. Bacterial production and growth efficiencies: direct measurements on riverine aggregates. Limnol. Oceanogr. **45**:436-445.

Grossart, H. P. and M. Simon. 1998. Bacterial colonization and microbial decomposition of limnetic organic aggregates (lake snow). Aquat. Microb. Ecol. **15**:127-140.

Hamadeh, H. and C.A. Afshari. 2000. Gene chips and functional genomics. Am. Sci. 88:508-515.

Heidelberg, J.F. and 31 co-authors. 2000. DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. Nature 406:477-486.

Hood, M. A., and P. A. Winter. 1997. Attachment of *Vibrio cholerae* under various environmental conditions and to selected substrates. FEMS Microbiol. Ecol. **22**:215-223.

Huq, A., I. N. G. Rivera, and R. R. Colwell. 2000. Epidemiological significance of viable but nonculturable microorganisms. Pages 301-323 *in* R. R. Colwell and D. J. Grimes, editors. Nonculturable Microoganisms in the Environment. ASM Press, Washington, DC.

Huq. A., R. R. Colwell, R. Rahman, A. Ali, M. A. R. Chowdhury, S. Parveen, D. A. Sack, and E. Russek-Cohen. 1990. Detection of *vibrio cholerae* O1 in the aquatic environment by fluorescent-monoclonal antibody and culture methods. Appl. and Environ. Microbiol. **56**:2370-2373.

Huq, A., E. Small, P. West, and R. R. Colwell. 1984a. The role of planktonic copepods in the survival and multiplication of *Vibrio cholerae* in the environment. Pages 521-534 *in* R. R. Colwell, editor. Vibrios in the Environment. John Wiley & Sons, New York, NY.

Huq, A., P. A. West, E. B. Small, M. I. Huq, and R. R. Colwell. 1984b. Influence of water temperature, salinity, and pH on survival and growth of toxigenic *Vibrio scholerae* serovar O1 associated with live copepods in laboratory microcosms. Appl. and Environ. Microbiol. **48**:420-424.

Huq, A., E. B. Small, P. A. West, M. I. Huq, R. Rahman, and R. R. Colwell. 1983. Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. Appl. Environ. Microbiol. **45**:275-283.

Huq, A., B. Xu, M. A. R. Chowdhury, M. S. Islam, R. Montilla, and R. R. Colwell. 1996. A simple filtration method to remove plankton-associated *Vibrio cholerae* in raw water supplies in developing countries. Appl. Environ. Microbiol. **62**:2508-2512.

Islam, M. S., M. J. Alam, A. Begum, Z. Rahim, A. Felsenstein, and M. J. Albert. 1996. Occurrence of culturable vibrio cholerae 0139 with ctx gene in various components of the aquatic environment in Bangladesh. Tran. Royal Soc. Trop. Med. Hyg. **90**:128.

Islam, M. S., B. S. Drasar, and D. J. Bradley. 1988. Survival and attachment of toxigenic *Vibrio cholerae* O1 in association with four marine algae. Bangladesh J. Microbiol. **5**:41-44.

Islam, M. S., B. S. Drasar, and D. J. Bradley. 1989. Attachment of toxigenic *Vibrio cholerae* 01 to various freshwater plants and survival with a filamentous green alga, *Rhizoclonium fontanum*. J. Trop. Med. Hyg. **92**:396-401.

Islam, M. S., B. S. Drasar, and D. J. Bradley. 1990. Long-term persistence of toxigenic *Vibrio cholerae* 01 in the mucilaginous sheath of a blue-green alga, *Anabaena variabilis*. J. Trop. Med. Hyg. **93**:133-139.

Islam, M. S., B. S. Drasar, and R. B. Sack. 1993. The aquatic environment as a reservoir of *Vibrio cholerae*: a review. J. Diarrhoeal Dis. Res. **11**:197-206.

Islam, M. S., B. S. Drasar, and R. B. Sack. 1994a. The aquatic flora and fauna as reservoirs of Vibrio cholerae: a review. J. Diarrhoeal Dis Res. **12**:87-96.

Islam, M., B. S. Drasar, and R. B. Sack. 1994b. Probable role of blue-green algae in maintaining endemicity and seasonality of cholera in Bangladesh: a hypothesis. J. Diarrhoel Dis. Res. **12**:245-256.

Islam, M. S., Z. Rahim, M. J. Alam, S. Begum, S. M. Moniruzzaman, A. Umeda, K. Amako, M. J. Albert, R. B. Sack, A. Huq, and R. R. Colwell. 1999. Association of *Vibrio cholerae* O1 with the cyanobacterium, *Anabaena* sp., elucidated by polymerase chain reaction and transmission electron microscopy. Trans. of Royal Soc. Trop. Med. Hyg. **93**:36-40.

Jensen, P. R., C. A. Kauffman, and W. Fenical. 1996. High recovery of culturable bacteria from the surfaces of marine algae. Marine Biol. **126**:1-7.

Langenheder, S., and K. Jürgens. 2001. Regulation of bacterial biomass and community structure by metazoan and protozoan predation. Limnol. Oceanogr. **46**:121-134.

Legendre, P. and L. Legendre. 1998. Numerical Ecology, 2nd ed. Elsevier Press, New York.

Linder, K., and J. D. Oliver. 1989. Membrane fatty acid and virulence changes in the viable but nonculturable state of *vibrio vulnificus*. Appl. Environ. Microbiol. **55**:2837-2842.

Lowenhaupt, E., A. Huq, R. R. Colwell, A. Adingra, and P. R. Epstein. 1998. Rapid detection of *Vibrio cholerae* O1 in west Africa. Lancet **351**:34-34.

McCarthy, S. A. 1996. Effects of temperature and salinity on survival of toxigenic *Vibrio cholerae* O1 in seawater. Microbial Ecol. **31**:167-175.

McSpadden Gardener. B.B., and F.J. De Bruijn. 1998. Detection and isolation of novel rhizopine-catabolizing bacteria from the environment. Appl. Environ. Microbiol. 64:4944-4949.
Middelboe, M., M. Sondergaard, Y. Letarte, and N. H. Borch. 1995. Attached and free-living bacteria - production and polymer hydrolysis during a diatom bloom. Microbial Ecol. **29**:231-248.

Montgomery, G.M. 1991. Design and Analysis of Experiments. John Wiley & Sons, New York.

Mullerniklas, G., S. Schuster, E. Kaltenbock, and G. J. Herndl. 1994. Organic content and bacterial metabolism in amorphous aggregations of the Northern Adriatic sea. Limnol. Oceanog. **39**:58-68.

Nagasawa, S. 1989. Bacterial epibionts of copepods. Sciences Progress **73**:169-176.

Nair, G. B., B. L. Sarkar, S. P. De, M. K. Chakrabarti, R. K. Bhadra, and S. C. Pal. 1988. Ecology of *Vibrio cholerae* in the freshwater environs of Calcutta, India. Microbial Ecol. **15**:203-215.

Nalin, D. R. 1976. Cholera, copepods and chitinase. Lancet **2**:958.

Nalin, D. R., V. Daya, A. Reid, M. M. Levine, and L. Cisneros. 1979. Adsorption and growth of *Vibrio cholerae* on chitin. Infect. Immun. **25**:768-770.

Norton, J.M. and M.K. Firestone 1991. Metabolic status of bacteria and fungi in the rhizosphere on Ponderosa pine-seedlings. Appl. Environ. Microbiol. **57**:1661-1167.

Oppenheimer, J. R., M. G. Ahmed, A. Huq, K. A. Haque, A. K. M. Ashraf-Ul-Alam, K. M. S. Aziz, S. Ali, and A. S. M. Motaharul Haque. 1978. Limnological studies of three ponds in Dacca, Bangaldesh. Bangladesh J. Fish. **1**:1-28.

O'Toole, G., H. B. Kaplan, and R. Kolter. 2000. Biofilm formation as microbial development. Ann. Rev. of Microbiol. **54**:49-79.

O'Toole, G. A., and R. Kolter. 1998b. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol. Microbiol. **30**:295-304.

Pace, M. L., and J. J. Cole. 1996. Regulation of bacteria by resources and predation tested in whole lake experiments. Limnol. and Oceanog. **41**:1448-1460.

Power, K. and K. C. Marshall. 1988. Cellular growth and reproduction of marine bacteria on surface-bound substrate. Biofouling **1**:163-174.

Ravel, J., R. T. Hill, and R. R. Colwell. 1994. Isolation of a *Vibrio cholerae* transposon mutant with an altered viable but nonculturable response. FEMS Microbiol. Lett. **120**:57-62.

Reynolds, C. S. 1984. The Ecology of Freshwater Phytoplankton. Cambridge University Press, New York, NY.

Richmond, C. S., J. D. Glasner, R. Mau, H. Jin, and F. R. Blattner. 1999. Genome-wide expression profiling in *Escherichia coli* K-12. Nucl. Acids. Res. **27**:3821-3835.

Ruiz, G.M., T.K. Rawlings, F.C. Dobbs, L.A. Drake, T. Mullady, A. Huq, and R.R. Colwell. 2000. Global spread of microorganisms by ships. Nature 408:51.

Schlesinger, W.H. 1997. Biogeochemistry: an analysis of global change. 2nd ed. Academic Press, San Diego, CA.

Schneider, D. R., and C. D. Parker. 1982. Purification and characterization of the mucinase of *Vibrio cholerae* non-O1 in India and Bangladesh. Lancet **341**:1347.

Seabloom, E.W., A.G. van der Valk, and K.A. Moloney. 1998. The role of water depth and soil temperature in determining initial composition of wetland coenoclines. Plant Ecol. 138:203-216.

Singleton, F. L., R. W. Attwell, M. S. Jangi, and R. R. Colwell. 1982. Influence of salinity and organic nutrient concentration on survival and growth of *Vibrio cholerae* in aquatic microcosms. Appl. Environ. Microbiol. **43**:1080-1085.

Smith, D. C., G. F. Steward, R. A. Long, and F. Azam. 1995. Bacterial mediation of carbon fluxes during a diatom bloom in a mesocosm. Deep-Sea Res. **42**:75-97.

Sterner, R.W., T.H. Chrzanowski, J.J. Elser, *et al.* 1995. Sources of nitrogen and phosphorus supporting the growth of bacterioplankton and phytoplankton in an oligotrophic Canadian shield lake. Limnol. Oceanogr. 40:242-249.

Stevenson, L. H. 1978. The case for bacterial dormancy in aquatic systems. Microb. Ecol. **4**:127-133.

Tamplin, M. L., A. L. Gauzens, A. Huq, D. A. Sack, and R. R. Colwell. 1990. Attachment of *vibrio cholerae* serogroup 01 to zooplankton and phytoplankton of bangladesh waters. Appl. Environ. Microbiol. **56**:1977-1980.

Tao, H., C. Bausch, C. Richmond, F. R. Blattner, and T. Conway. 1999. Functional genomics: expression analysis of *Escherichia coli* growing on minimal and rich media. J. Bacteriol. **181**:6425-6440.

Threlkeld, S. T., D. A. Chiavelli, and R. L. Willey. 1993. The organization of zooplankton epibiont communities. Trends Ecol. Evol. **8**:317-321.

Turley, C. M., and P. J. Mackie. 1994. Biogeochemical significance of attached and free-living bacteria and the flux of particles in the NE Atlantic-Ocean. Marine Ecology-Progress Series **115**:191-203.

Turley, C. M., and E. D. Stutt. 2000. Depth-related cell-specific bacterial leucine incorporation rates on particles and its biogeochemical significance in the Northwest Mediterranean. Limnol. Oceanogr. **45**:419-425.

Unanue, M., I. Azua, J. M. Arrieta, A. Labirua-Iturbur, L. Egea, and J. Iriberri. 1998. Bacterial colonization and ectoenyzmatic activity in phytoplankton-derived model particles: cleavage of peptides and uptake of amino acids. Microbial Ecol. **35**:136-146.

Underwood, A.J. 1997. Experiments in Ecology: Their Logical Design and Interpretation Using Analysis of Variance. Cambridge University Press, New York.

Watnick, P. I., and R. Kolter. 1999. Steps in the development of *Vibrio cholerae* El Tor biofilm. Mol. Microbiol. **34**:586-595.

Wei, Y., J. M. Lee, C. Richmond, F. R. Blattner, J. A. Rafalski, and R. A. LaRossa. 2001. High-density microarray-mediated gene expression profiling of Escherichia coli. J. Bacteriol. **183**:545-556.

West, P. A., and J. V. Lee. 1982. Ecology of *Vibrio* species including *Vibrio cholerae* in natural waters of Kent, England. J. Appl. Bacteriol. **52**:435-448.

Willey, R. L., and S. T. Threlkeld. 1995. Changes in epibiotic burden during the intermolt of *Daphnia* determined by hypodermal retraction stages. Hydrobiol. **307**:213-219.

Xu, H.-S., N. Roberts, F. L. Singleton, R. W. Attwell, D. J. Grimes, and R. R. Colwell. 1982. Survival and viability of nonculturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment. Microbial Ecol. **8**:313-323.

Yildiz, F.H, N.A. Dolganov, G.K. Schoolnik. 2001. VpsR, a member of the response regulators of the two-component regulatory systems, is required for expression of vps biosynthesis genes and EPS (ETr)-associated phenotypes in *Vibrio cholerae* O1 El Tor. J. Bacteriol. 83:1716-26.

Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh through a training programme.

The investigators at all of three sites are involved in postgraduate and/or undergraduate education, as well as other community educational activities. For example, R. Taylor directs a training grant and coordinates activities and seminars that are integrated into the activities of the DEHS. Consultant G O'Toole will incorporate approaches and results from the proposed studies into the graduate course ("Emerging Model Systems in Microbiology: Using Molecular & Genetic Approaches to Study Complex Systems") he is developed as part of his NSF/CAREER award. K. Cottingham and R. Taylor are involved in many educational activities outside of the college that range from children's science programs, to a new high school experience planned for May, to international courses and workshops. D. Chiavelli and R. Taylor have participated as mentors in the Dartmouth Women in Science Program (partially supported by NSF). In addition, our proposed web site will post the first environmentally-relevant microarray data. Scientific papers will be written from the data generated from this project and will be published in joint authorship of the investigators in peer reviewed journals.

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization. (DO NOT EXCEED ONE PAGE)

In addition to electronic networking between project personnel, there will be additional mechanisms to share data and coordinate research efforts. Of particular note, Dartmouth has granted a leave of teaching and administrative duties to K. Cottingham so that she can take a mini-sabbatical in the labs of R. Taylor and A. Huq in the fall term of 2001. She plans to become formally trained in the technologies used in these two labs in order to optimize the means by which they are integrated into the ecological aims of this proposal. D. Chiavelli and A. Huq will make annual trips to the ICDDR,B to coordinate results of lab and field studies. A. Huq, in particular, has worked with the ICDDR,B on numerous occasions, and he will play a major role in coordinating the work of all three institutions. Importantly, this will involve arranging an annual meeting of all the major investigators. This meeting will be held in Baltimore and will be coordinated with the annual trip of S. Islam to the US.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name	Position	Date of Birth
Ronald K. Taylor	Professor, Microbiology and Immunology	

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
State University of New York, Buffalo	B.A.	1976	Cell and Molecular Biology
University of Maryland, Baltimore	Ph.D.	1984	Biological Sciences
Harvard Medical School	Postdoctor-al Research	1983-1986	Bacterial Pathogenesis

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. **(DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).**

1. Professor, Microbiology and Immunology, Dartmouth Medical School, 1997-present
2. Associate Professor, Microbiology and Immunology, Dartmouth Medical School, 1993-1997
3. Associate Professor, Microbiology and Immunology, University of Tennessee, Memphis, 1989-1993
4. Assistant Professor, Microbiology and Immunology, University of Tennessee, Memphis, 1986-1989
5. Postdoctoral Fellow (NIH), Microbiology and Molecular Genetics, Harvard Medical School, 1984-1986
6. Postdoctoral Fellow (Anna Fuller Fund), Microbiology and Molecular Genetics, HMS, 1983-1984

Bibliography

Chiavelli, D.A., Marsh, J.A., and R.K. Taylor. The mannose sensitive hemagglutinin of Vibrio cholerae promotes adherence to zooplankton. Submitted for publication.

Kirn, T.J., M.J. Lafferty, C.M.P. Sandoe, and R.K. Taylor. 2000. Delineation of pilin domains that mediate direct TCP interactions required for bacterial association into microcolonies and intestinal colonization by Vibrio cholerae. Mol. Microbiol. 35:896-910.

Murley, Y.M., P.A. Carroll, K. Skorupski, R.K. Taylor, and S.B. Calderwood. 1999. Differential transcription of the tcpPH operon confers biotype-specific control of the Vibrio cholerae ToxR virulence regulon. Infect. Immun. 67: 5117-5123.

Marsh, J.W. and R.K. Taylor. 1999. Genetic and transcriptional analyses of the Vibrio cholerae manose sensitive hemagglutinin type 4 pilus gene locus. J. Bacteriol. 181:1110-1117.

DiRita, V.J., M. Neeley, R.K. Taylor, and P.M. Bruss. 1996. Differential expression of the ToxR regulon in classical and El Tor biotypes of Vibrio cholerae in due to specific control over toxT expression. Proc. Natl. Acad. Sci. USA. 93: 7991-7995.

Nye, M.B., Pfau, J.K., Skorupski, K., and R.K. Taylor. 2000. Vibrio cholerae H-NS silences virulence gene expression at multiple steps in the ToxR regulatory cascade. J. Bacteriol. 182: 4295-4303.

LaPinte, C.F. and R.K. Taylor. 2000. The type 4 prepilin peptidases comprise a novel family of aspartie acid protease. J.Biol. Chem. 275:1502-1510.

Marsh, J.W. and R.K. Taylor. 1998. Identification of the Vibrio cholerae type 4 prepilin peptidase required for cholera toxin secretion and pilus formation. Mol. Microbiol. 29:1481-1492.

Tacket, C.O., R.K. Taylor, G. Losonsky, J.P. Nataro, J.B. Kaper, and M.M. Levine. 1998. Investigation of the roles of TCP and MSHA pili in the pathogenesis of Vibrio cholerae O139 infection. Infect. Immun. 66:692-695.

SYNERGISTIC ACTIVITIES

Training Grant Principle Investigator: I am the PI of an NIH training grant entitled,"Host-Microbe Interactions". It currently supports two graduate students and two postdoctoral fellows selected from among 14 laboratories at DMS and DC that conduct related research.

Technology Development: My laboratory has developed a number of techniques that are used world-wide for the genetic analysis of bacteria. These include transposon mutagenesis systems, allelic exchange systems, site-directed mutagenesis methods, and microarray analysis.

Meeting Organizer: I am the co-organizer of the biannual meeting of Microbial Pathogenesis and Host Response, held at Cold Spring Harbor Laboratory. I have been a co-organizer since the meeting's inception in 1997.

International Courses: I have been an instructor for several international courses that involve genetic approaches to the analysis of bacteria. These include the Advanced Bacterial Genetics Course at CSHL from 1991-1995, a UNIDO Advanced Genetics Course in Trieste, Italy from 1990-1996, and most recently the Gulbenkian Foundation International Advanced Course on Molecular Genetics of Bacteria held in Porto and Braga, Portugal in 1997 and 2001.

Center for Environmental Health Sciences at Dartmouth: I am a member of this new center at Dartmouth that serves to foster the initiation of collaborative projects having to do with environmentally related research. I hope to promote the integration of molecular and genetic approaches into environmental studies involving microbial populations.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name	Position	Date of Birth
Md. Sirajul Islam	Environmental Microbiologist	01-01-1952

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
Department of Botany, University of Dhaka, Bangladesh	M.Sc.	1976	Bacteriology, Microbiology, Limnology & Ecology
Department of Tropical Hygiene, London School of Hygiene and Tropical Medicine, UK.	Ph.D.	1987	Environmental Disease Transmission

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. **(DO NOT EXCEED TWO PAGES. USE CONTINUATION SHEETS).**

Environmental Microbiologist, Laboratory Sciences Division, ICDDR,B., 2001-present
Scientist, Laboratory Sciences Division, ICDDR,B., 1994-2001
Associate Scientist, Laboratory Sciences Division, ICDDR,B, 1992-94
Assistant Scientist, Laboratory Sciences Division, ICDDR,B, 1988-92
Ph.D. Scholar, London University, United Kingdom, 1983-87
Senior Research Officer, Training Branch, ICDDR,B, 1979-83
Research Officer, Microbiology Branch, Matlab, ICDDR,B, 1978-79
Research Scholar, National Council of Science and Technology, Government of Bangladesh, 1977-77
Research Scholar, Asiatic Society of Bangladesh, 1977-77

Bibliography

Islam, M.S., Siddika, A., Khan, M.N.H., Goldar, M.M., Sadique, M.A., Kabir, A.N.M.H., Huq, A., and Colwell, R.R. (2001). Microbiological analyis of tube-well water in a rural area of Bangladesh. Applied and Environmental Microbiology. 67: 3328-3330.

Islam, M.S., Begum, A., Khan, S.I., Sadique, M.A., Khan, M.N.H., Albert, M.J., Yunus, M., Huq, A. and Colwell, R.R. (2000). Microbiology of pond ecosystems in rural Bangladesh: its public health implications. The International Journal of Environmental Studies. 58:33-46

Islam, M.S., Rahim, Z., Alam, M.J., Begum, S., Moriruzzaman, S.M., Umeda, A., Amako, K., Albert, M.J., Sack, R.B., Huq, A. and Colwell, R.R. (1999). Association of *Vibrio cholerae* 1 with the Cyanobacterium, *Anabaena* sp. Elucidated by PCR and Transmission Electron Microscopy. Transactions of the Royal Society of Tropical Medicine & Hygiene. 93:36-40

Islam, M.S., Hasan, M.K., Miah, M.A., Yunus, M., Zaman, K. and Albert, M.J. (1994). Isolation of *Vibrio cholerae* 0139 Synonym Bengal from the Aquatic Environment of Bangladesh: Implications for Disease Transmission. Applied and Environmental Microbiology. **60** : 1684-1686.

Islam, M.S., Alam, M.J., Begum, A., Rahim, Z., Felsenstein, A. and Albert, M.J. (1996). Occurrence of culturable *Vibrio cholerae* O139 Bengal with *ctx* gene in various components of aquatic environment in Bangladesh. Transactions of the Royal Society of Tropical Medicine and Hygiene. 90: 128.

Islam, M.S., Drasar, B.S., Albert, M.J., Sack, R.B., Huq, A. and Colwell, R.R. (1997). Toxigenic *Vibrio cholerae* in the environment - A minireview. Tropical Diseases Bulletin. 94:R1-R11

Islam, M.S., Alam, M.J., Miah, M.A., Felsenstein, A. and Sack, R.B. (1995). Detection of non-culturable *Vibrio cholerae* O139, by PCR and fluorescent antibody methods, in laboratory microcosms. World Journal of Microbiology and Biotechnology. 11: 597-598.

Islam, M.S., Siddique, A.K.M., Salam, A., Akram, K., Zaman, K., Fronczak, N. and Laston, S. (1995). Microbiological investigation of diarrhoea epidemics among Rwandan refugees in Goma, Zaire. Transactions of the Royal Society of Tropical Medicine and Hygiene. 89: 506.

Islam, M.S., Alam, M.J. and Khan, S.I. (1995). Occurrence and distribution of Culturable *Vibrio cholerae* 01 in aquatic environments of Bangladesh. International Journal of Environmental Studies. 46 : 217-223

Islam, M.S., Hasan, M.K., Miah, M.A., Qadri, F., Yunus, M.,Sack, R.B. and Albert, M.J. (1993). Isolation of *Vibrio cholerae* 0139 Bengal from water in Bangladesh. Lancet. 342:430. Islam, M.S., Drasar, B.S. and Sack, R.B. (1994). Probable role of blue-green algae in maintaining endemicity and seasonality of cholera in Bangladesh : A hypothesis. Journal of Diarrhoeal Diseases Research. 12: 245-256.

Islam, M.S., Drasar, B.S. and Bradley, D.J. (1990) Long term persistence of toxigenic *Vibrio cholerae* 01 in the mucilaginous sheath of a blue green alga, *Anabaena variabilis*. The Journal of Tropical Medicine and Hygiene. 93,133-139.

SYNERGISTIC ACTIVITIES

In addition to research, I also actively participate as course Director, course Co-Director and faculty member in various courses that are offered by ICDDR,B from time to time. I have conducted four International courses on Laboratory aspects of Diarrhoeal Diseases as course Director and four as Co-course Director. I have also conducted 8 national courses on microbiological aspects of diarrhoeal diseases as course Director. So far 24 students have completed their M.Sc. theses under my supervision. I am also supervising a M. Phil and four M.Sc. students at present. *The responsibilities with Training Branch are in addition to the Scientific investigations that I am doing in the* Laboratory Sciences Division. I am also coordinating the post graduate students training programme of LSD.

I also shoulder the administrative responsibilities of the Laboratory Sciences Division as Acting Division Director from time to time.

I am the Editor-in-Chief of the News Letter published by the Bangladesh Environmental Society (BES).

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name	Position	Date of Birth
Anwar Huq, Ph.D.	Associate Professor	March 16, 1951

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
University of Karachi, Karachi, Pakistan	B.Sc. (Honors)	1973	Zoology
University of Karachi, Karachi, Pakistan	M.Sc.	1973	Marine Zoology
University of Maryland, Maryland, USA	Ph.D.	1984	Microbiology

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees.

1975-1976	Research Assistant, Johns Hopkins University, ICMRT, Dhaka, Bangladesh.
1976-1980	Research Officer, International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B).
1980-1984	Faculty Research Assistant, University of Maryland
1984-1987	Assistant Scientist and Manager, Laboratory Services Dept., ICDDR,B.
1987-1992	Associate Scientist, ICDDR,B
1986-1989	Head, Environmental Microbiology Laboratory, ICDDR,B
1987-1992	Head, Research Microbiology Branch of ICDDR,B
1989-1995	Research Assistant Professor, Department of Microbiology, University of Maryland, College Park, Maryland.
1995-1997	Research Associate Professor, Department of Microbiology, University of Maryland, College Park, Maryland.
1997-2001	Research Associate Professor, University of Maryland Biotechnology Institute, Center of Public Issues in Biotechnology, Maryland
2001-present	Associate Professor, University of Maryland Biotechnology Institute, Center of Marine Biotechnology Biotechnology, Maryland

Research Focus

Microbial ecology, environmental transmission of pathogens, particularly *Vibrio cholerae* and related organisms. Survival and multiplication of bacterial pathogens in the environment and detection of these pathogens both in culturable and viable but nonculturable state. Study on the effect of climate change on marine and estuarine microorganisms and application of aerospace technology for detection of pathogens, global prediction and intervention of cholera outbreak.

International Appointments

- Elected member of the International Congress on Systemic Bacteriology, Subcommittee on Taxonomy of *Vibrionaceae* of the International Union of Microbiological Societies, 1988 - present.
- Director, University of Maryland Biotechnology Institute-UNESCO World Network of Microbiological Resources Centers (MIRCEN). 1998 – present.

Membership In Professional Societies

Bangladesh Society of Microbiologists (BSM), 1977 - Life member.
Bangladesh Association for the Advancement of Science (BAAS), 1978-91.
American Society for Microbiology (ASM), 1980 - Present.
Bangladesh Society for Immunology (BSI), 1991- Life member.
American Association for Advancement of Science (AAAS), 1996 - Present.
Sigma Xi - The Scientific Research Society, 1991-1997.
Washington-DC Branch of American Society for Microbiology, 1993-1996.
American Society of Tropical Medicine and Hygiene, 1999 – Present

Awards and Fellowships:

- Fellow of the American Academy of Microbiology, 1999- Present.

- Certificate of Appreciation in recognition of outstanding contributions to the sixth NASA worldwide internet-broadcast seminar series on "emerging Diseases", January-June, 2001, by the National Aeronautics and Space Administration, Washington D. C.,
- Certificate of Merit in recognition of contributions to nursing research and to the nation's health by the Friends of the National Institute of Nursing Research, National Institutes of Health, Bethesda, Maryland, September, 2000.

Bibliography

List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. **(DO NOT EXCEED TWO PAGES, USE CONTINUATION SHEETS).**

Selected Publications: Published or in press (Author or co-author of 71 papers, 24 chapters in books, proceedings and 108 abstracts.)

- Colwell, R.R., R. Seidler, J. Kaper, S.W. Joseph, S. Garges, H. Lockman, D. Maneval, H. Bradford, N. Roberts, E. Remmers, **A. Huq**. 1981. Occurrence of *V. cholerae* serotype O1 in Maryland and Louisiana Estuaries. *Appl. Environ. Microbiol.* 41:555-558.
- **Huq, A.**, E.B. Small, P.A. West, M.I. Huq, R. Rahman, R.R. Colwell. Ecology of *V. cholerae* with special reference to planktonic crustacean copepods. *App. Environ. Microbiol.* 48:420-424.
- **Huq, A.** 1986. Vibrios in abudance: a growing genus. *J. Diarrhoeal Dis. Res.* 4(4):209-210.
- **Huq, A.**, R.R. Colwell, R. Rahman, A. Ali, M.A.R. Chowdhur, Salina Parveen, D.A. Sack and E. Russek-Cohen. 1990. Occurrence of *V. cholerae* in the Aquatic Environment Measured by Fluorescent Antibody and Culture Method. *Appl. Environ. Microbiol.* 56:2370-73.
- Colwell, R.R., J.A.K. Hasan, **A. Huq**, L. Loomis, Mark L. Tamplin, R.J. Seibling, M. Torres, S. Galvez, S. Islam and D. Bernstein. 1992. Development and evaluation of a rapid, simple and sensitive monoclonal antibody-based co-agglutination test for direct detection of *V. cholerae* 01. *FEMS Microbiol. Letters.* 97:215-220.
- **Huq, A.**, J.A.K. Hasasn, G. Losonsky and R.R. Colwell. 1994. Occurrence of *Toxigenic V. cholerae* O1 and *V. cholerae* non-O1 in professional divers and dive sites in the United States, Ukraine, and Russia. *FEMS Microbiol. Letters.* 120:137-142.
- **Huq, A.**, R.R. Colwell, M.A.R. Chowdhury, B. Xu, S.M. Moniruzzaman, M.S. Islam, M. Yunus and M.J. Albert. 1995. Co-existence of *V. cholerae* O1 and O139 Bengal in plankton in Bangladesh. *The Lancet.* 345:1249.
- **Huq, A.** and R.R. Colwell. 1995. Vibrios in marine and estuarine environment. *J. Mar. Biotechnology.* 3:60-63.
- **Huq, A.** and R.R. Colwell. 1996. Vibrios in the Environment: Tracking of *Vibrio cholerae.* In *Marine Ecosystem and Health.* P.A. Epstein (ed.). Blackwell Scientific Publications. Ecosystems Health, Vol. 2, No. 3, pp. 198-214.
- **Huq, A.** and R.R. Colwell. 1996. Environmental factors associated with emergence of disease: special reference to cholera. *Eastern Mediterranean Hlt. J.* 2:37-45.
- M.A.R., Chowdhury, Montilla, R., **A. Huq**, B. Xu and R.R. Colwell. 1996. Serogroup conversion from *Vibrio cholerae* non-O1 to *V. cholerae* O1: Effect of growth state of cells, temperature and salinity. Can. J. Microbial. 42:87-93.
- **Huq, A.**, and R.R. Colwell. 1996. A microbiological paradox: viable but non-culturable bacteria with special reference to *Vibrio cholerae*. J. Food Protection. 59:96-101.
- **Huq, A.**, R.R. Colwell, M.A.R. Chowdhury, B. Xu and R. Montilla. 1996. A simple filtration method for removal of *Vibrio cholerae* associated with planktonic copepods. Appl. Environ. Microbiol. 62:2508-2512.
- Chun, J., **A. Huq**, and R. R. Colwell. 1999. Analysis of 16S-23S rRNA Intergenic Spacer Regions of *Vibrio cholerae* and *Vibrio mimicus*.. Appl. Environ. Microbiol. 65:2202-2208.
- Jiang, S. C., N. Choopun, V. Louis, **A. Huq**, R. R. Colwell. 2000. Genetic diversity of *Vibrio cholerae* in Chesapeake Bay determined by Amplified Fragment Length Polymorphism Fingerprinting. Appl. Environ. Microbiol. 66:140-147.
- Lobitz, B., L. Beck, **A. Huq**, B. Wood, and R.R. Colwell. 1999. Climate and Infectious Disease: Use of remote sensing for detection of *V. cholerae* by indirect measurement. Proc. Natl. Acad. Sc. 97:1438-1443.
- Jiang, S. C., M. Matte, G. Matte, **A. Huq**, R. R. Colwell. 2000. Genetic Diversity of Clinical and Environmental Isolates of *Vibrio* Determined by Amplified Fragment Length Polymorphism Fingerprinting. Appl. Environ. Microbiol. 66:148-153.
- **Huq, A.**, I. Rivera, and R. R. Colwell. 2000. Epidemiological Significance of Viable But nonculturable Microorganisms. *In* Nonculturable Organisms in the Environment. R. R. Colwell and J. Grimes (*Ed*). ASM Publishers, Washington, D. C. Chapter 17, pp. 301-323.
- Ruiz, G. M., T. K. Rawlings, F. C. Dobbs, L. A. Drake, T. Mullady, **A. Huq** and R. R. Colwell. 2000. Worldwide transfer of microorganisms by ships. The Nature. 408:49-50.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

Name	Position	Date of Birth
Deborah A. Chiavelli	Post Doctoral Fellow	

Academic Qualifications (Begin with baccalaureate or other initial professional education)

Institution and Location	Degree	Year	Field of Study
Dartmouth Medical School, USA	Ph.D.	2000	Association of *V. cholerae* with zooplankton

Research and Professional Experience

Concluding with the present position, list, in chronological order, previous positions held, experience, and honours. Indicate current membership on any professional societies or public committees. List, in, chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. **(DO NOT EXCEED TWO PAGES. USE CONTINUATION SHEETS).**

1. Graduate Student of the Department of Microbiology and Immunology, Dartmouth Medical School, USA.
2. Post Doctoral Fellow of the Department of Microbiology and Immunology, Dartmouth Medical School, USA.

Bibliography

Chiavelli, D.A., J.W. Marsh, and R.K. Taylor. The mannose-sensitive hemagglutinin of *Vibrio cholerae* promotes adherence to zooplankton. Appl. Environ. Microbiol. Submitted.

Threlkeld, S. T., D. A. Chiavelli, and R. L. Willey. 1993. The organization of zooplankton epibiont communities. Trends Ecol. Evol. **8**:317-321.

APPENDIX

International Centre for Diarrhoeal Disease Research, Bangladesh
Voluntary Consent Form

Title of the Research Project: **ENVIRONMENTAL PERSISTENCE OF *Vibrio cholerae***

Principal Investigator: DR. MD. SIRAJUL ISLAM (Local)

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. For children, consents must be obtained from their parents or legal guardians. The subject must indicate his/ her acceptance of participation by signing or thumb printing on this form.

Not Applicable

Signature of Investigator/ or agents
Date:

Signature of Subject/ Guardian
Date:

SUMMARY PROPOSAL BUDGET — YEAR 1

					FOR NSF USE ONLY	
ORGANIZATION: International Centre for Diarrhoeal Disease Research, Bangladesh					PROPOSAL NO.	DURATION (months) Proposed / Granted
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR: Sirajul Islam					AWARD NO.	

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-mos. CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Sirajul Islam - Scientist	3.19	0.00	0.00	$ 26,892	$
2.					
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)	3.19	0.00	0.00	26,892	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (2) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	12.00	0.00	0.00	8,640	
3. (0) GRADUATE STUDENTS				0	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				35,532	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				0	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				35,532	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				3,000	
2. FOREIGN				3,400	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				15,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				24,858	
TOTAL OTHER DIRECT COSTS				39,858	
H. TOTAL DIRECT COSTS (A THROUGH G)				81,790	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) (Rate: , Base:)					
TOTAL INDIRECT COSTS (F&A)				0	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				81,790	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.D.7.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 81,790	$
M. COST SHARING PROPOSED LEVEL $ 0	AGREED LEVEL IF DIFFERENT $				

PI / PD TYPED NAME & SIGNATURE*	DATE	FOR NSF USE ONLY		
Sirajul Islam M. S. Islam	19.7.2001	INDIRECT COST RATE VERIFICATION		
ORG. REP. TYPED NAME & SIGNATURE*	DATE	Date Checked	Date Of Rate Sheet	Initials - ORG
Shamima Moin	19 July 2001			

NSF Form 1030 (10/99) Supersedes all previous editions

1 *SIGNATURES REQUIRED ONLY FOR REVISED BUDGET (GPG III.B)

Shamima Moin
Controller, Budget & Costing
& Acting Chief Finance Officer
International Centre for Diarrhoeal
Disease Research, Bangladesh
Mohakhali, Dhaka-1212

SUMMARY PROPOSAL BUDGET — YEAR 2

	FOR NSF USE ONLY		
ORGANIZATION: International Centre for Diarrhoeal Disease Research, Bangladesh	PROPOSAL NO.	DURATION (months) Proposed	Granted
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR: Sirajul Islam	AWARD NO.		

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-mos. CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Sirajul Islam - Scientist	3.19	0.00	0.00	$ 28,237	$
2.					
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)	3.19	0.00	0.00	28,237	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (2) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	12.00	0.00	0.00	9,072	
3. (0) GRADUATE STUDENTS				0	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				37,309	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				0	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				37,309	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				3,000	
2. FOREIGN				3,536	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				15,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				23,961	
TOTAL OTHER DIRECT COSTS				38,961	
H. TOTAL DIRECT COSTS (A THROUGH G)				82,806	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) (Rate: , Base:)					
TOTAL INDIRECT COSTS (F&A)				0	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				82,806	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.D.7.J.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 82,806	$
M. COST SHARING PROPOSED LEVEL $ 0 — AGREED LEVEL IF DIFFERENT $					

PI / PD TYPED NAME & SIGNATURE*	DATE	FOR NSF USE ONLY — INDIRECT COST RATE VERIFICATION		
Sirajul Islam *M. S. Islam*	19.7.2001			
ORG. REP. TYPED NAME & SIGNATURE*	DATE	Date Checked	Date Of Rate Sheet	Initials - ORG
Shamima Moin	19 July 2001			

NSF Form 1030 (10/99) Supersedes all previous editions — 2 *SIGNATURES REQUIRED ONLY FOR REVISED BUDGET (GPG III.B)

Shamima Moin
Controller, Budget & Costing & Acting Chief Finance Officer
International Centre for Diarrhoeal
Disease Research, Bangladesh
Mohakhali, Dhaka-1212

SUMMARY PROPOSAL BUDGET YEAR 3

ORGANIZATION	FOR NSF USE ONLY		
International Centre for Diarrhoeal Disease Research, Bangladesh	PROPOSAL NO.	DURATION (months)	
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR		Proposed	Granted
Sirajul Islam	AWARD NO.		

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-mos. CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Sirajul Islam - Scientist	3.19	0.00	0.00	$ 29,648	$
2.					
3.					
4.					
5.					
6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)	3.19	0.00	0.00	29,648	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (2) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	12.00	0.00	0.00	9,526	
3. (0) GRADUATE STUDENTS				0	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				39,174	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				0	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				39,174	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				3,000	
2. FOREIGN				3,677	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				15,350	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				25,075	
TOTAL OTHER DIRECT COSTS				40,425	
H. TOTAL DIRECT COSTS (A THROUGH G)				86,276	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) (Rate: , Base:)					
TOTAL INDIRECT COSTS (F&A)				0	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				86,276	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.D.7.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 86,276	$
M. COST SHARING PROPOSED LEVEL $ 0 — AGREED LEVEL IF DIFFERENT $					

PI / PD TYPED NAME & SIGNATURE*	DATE	FOR NSF USE ONLY		
Sirajul Islam *M. S. Islam*	19.7.2001	INDIRECT COST RATE VERIFICATION		
ORG. REP. TYPED NAME & SIGNATURE*	DATE	Date Checked	Date Of Rate Sheet	Initials - ORG
Shamima Moin	19 July 2001			

NSF Form 1030 (10.99) Supersedes all previous editions

3 *SIGNATURES REQUIRED ONLY FOR REVISED BUDGET (GPG III.B)

Shamima Moin
Controller, Budget & Costing
Acting, Chief Finance Officer
International Centre for Diarrhoeal
Disease Research, Bangladesh
Mohakhali, Dhaka-1212

SUMMARY PROPOSAL BUDGET

Cumulative

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months) Proposed	Granted
AWARD NO.		

ORGANIZATION

International Centre for Diarrhoeal Disease Research, Bangladesh

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR

Sirajul Islam

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1. Sirajul Islam - Scientist	9.57	0.00	0.00	$ 84,777	$
2.					
3.					
4.					
5.					
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	0	
7. (1) TOTAL SENIOR PERSONNEL (1 - 6)	9.57	0.00	0.00	84,777	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (0) POST DOCTORAL ASSOCIATES	0.00	0.00	0.00	0	
2. (6) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	36.00	0.00	0.00	27,238	
3. (0) GRADUATE STUDENTS				0	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				112,015	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				0	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				112,015	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)				9,000	
2. FOREIGN				10,613	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				45,350	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				73,894	
TOTAL OTHER DIRECT COSTS				119,244	
H. TOTAL DIRECT COSTS (A THROUGH G)				250,872	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)					
TOTAL INDIRECT COSTS (F&A)				0	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				250,872	
K. RESIDUAL FUNDS (IF FOR FURTHER SUPPORT OF CURRENT PROJECTS SEE GPG II.D.7.j.)				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				$ 250,872	$

M. COST SHARING PROPOSED LEVEL $ 0 AGREED LEVEL IF DIFFERENT $

PI / PD TYPED NAME & SIGNATURE*	DATE	FOR NSF USE ONLY		
Sirajul Islam *M. S. Islam*	19.7.2001	INDIRECT COST RATE VERIFICATION		
ORG. REP. TYPED NAME & SIGNATURE*	DATE	Date Checked	Date Of Rate Sheet	Initials - ORG
Shamima Moin	19 July 2001			

NSF Form 1030 (10/99) Supersedes all previous editions

*SIGNATURES REQUIRED ONLY FOR REVISED BUDGET (GPG III.B)

Shamima Moin
Controller, Budget & Costing *& Acting, Chief Finance Officer*
International Centre for Diarrhoeal
Disease Research, Bangladesh
Mohakhali, Dhaka-1212

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item. Justify use of man power, major equipment, and laboratory services.

The 25% salary of the local PI and 2 Research Officers have been budged in this project for 3 years. The expenses for local travel to Matlab, Chandpur and Dhaka has been budgeted. The expenses for doing microbiological assays have been included as reagents and chemical costs.

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration. (DO NOT EXCEED ONE PAGE FOR EACH INVESTIGATOR)

TAYLOR, R.K.

ACTIVE

1 RO1 AI 25096-15 (R.K. Taylor) 05/01/97-04/30/2002 35%
NIH /NIAID $ 211,000
Genetic Determinants of Virulence in V*ibrio cholerae*

The major goals of this grant are to characterize the molecular mechanisms that mediate colonization by type 4 piliated bacteria, to understand the biogenesis of type 4 pili, and to use certain type 4 piliated derivatives of as live delivery vehicles for vaccination with antigens specific to other mucosal infectious diseases.

AI 47383-01 (W.F. Wade) 04/01/00-03/31/2005 20%
NIH $200,000
Vibrio cholerae TCP and LPS Subunit Vaccine, Epitopes and Efficacy

This grant proposal focuses on new approaches to develop a cholera subunit vaccine formulation based on the current understanding of *V. cholerae* colonizatioin, pathogenesis, and human immune responses to infection.

Research Grant (R.K. Taylor) 04/01/2001-03/31/2003 15%
Cystic Fibrosis Foundation $60,000
Novel P. aeruginosa Antibiotic Targets and Compounds

This project includes testing the hypothesis that PilD of P. aeruginosa is analogous to other bacterial Type Four Leader Peptidases and functions via the same catalytic mechanism of the TFPPs of *V. cholerae*. The proposal focuses on testing PilD inhibitors using high throughout screens and solving the crystal structure of PilD to facilitate rational drug design.

PENDING

1 R01 AI 39654-06-10 (R.K. Taylor) 06/01/2001-05/31/2006 20%
NIH/NIAID $ 250,000 requested
Hierarchy Within Enviornmental Regulons

The major goals of this project are to discern mechanisms by which the environment within the human host is responsible for inducing expression of specific genes of infecting bacteria. The model system being analyzed is the *Vibrio cholerae* ToxR regulon.

Other Support

Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration. (DO NOT EXCEED ONE PAGE FOR EACH INVESTIGATOR)

ISLAM, M.S.
ACTIVE

NIAID/NIH	01/09/96-31/08/2001	60%
Epidemiology and ecology of *V. cholerae* in Bangladesh.	$ 574,317	

The major goals of this grant are to find out the role of zooplankton, phytoplankton and physicochemical parameters in maintaining endemicity and seasonality of cholera in Bangladesh.

NINR/NIH	01/08/98-31/09/2002	20%
A simple water filtration for cholera intervention	$545,955	

The major goals of this grant are to reduce the incidence of cholera and diarrhoeal diseases by filtering household water collected from various surfaces water sources e.g., ponds, lakes, rivers etc. and passing through 8 folds old saree (cloth) material as a filtering device.

NIH	01/02/01-31/01/2002	10%
Biofilm formation by *Vibrio cholerae* O1 in Bangladeshi natural aquatic habitats	$26,721	

The major goals of this grant are to isolate the *V. cholerae* O1 from the biofilm, form on plexiglass made devices which have been immerged at different depths of aquatic environment in Bangladesh. This device is being used to attract various zooplankton and phytoplankton to form biofilm along with other organisms and is also used as a trap for capturing *V. cholerae* O1 from the aquatic environment.

Other Support

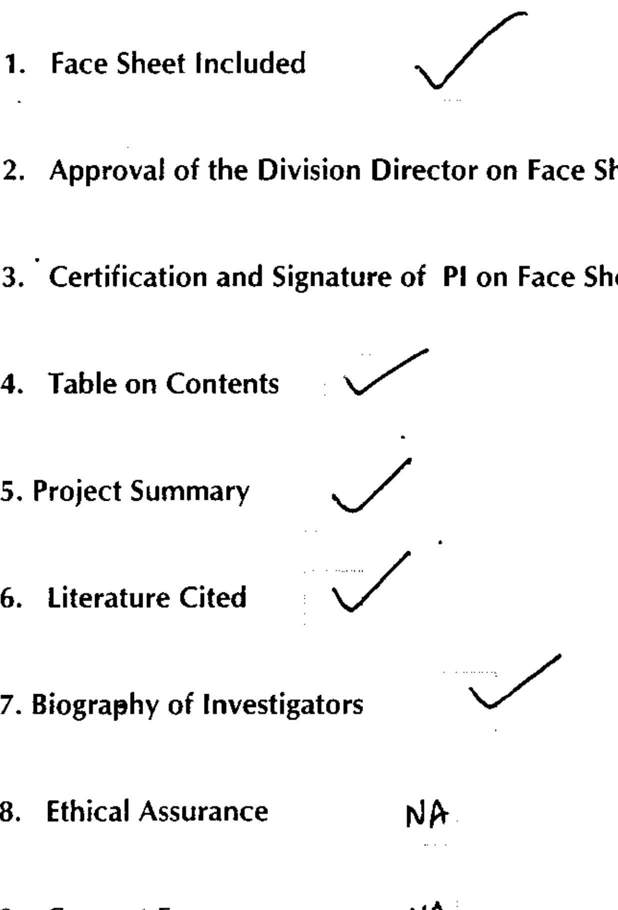
Describe sources, amount, duration, and grant number of all other research funding currently granted to PI or under consideration. (DO NOT EXCEED ONE PAGE FOR EACH INVESTIGATOR)

HUQ, A.

- Clinical, Environmental & Molecular Epidemiology of Cholera. Funded by NIH/JHU. September 1, 1996 to August 31, 2001. Total funds $746,567. Grant Number 1RO1A139129-01
- Application of Remote Sensing for the Prediction of cholera outbreak. Funded by NASA. April 22, 1998 to July 31, 2002. Total funds $141,070. Grant Number NAG-2-1195
- Effects of Environmental change and pollution on *V. cholerae*, a bacterium autochthonous to the aquatic environment. Funded by the Wallenberg Foundation. September 22, 1997 to September 21, 2001. Total funds $132,000.
- A Simple Water Filtration for Cholera Intervention.. Funded by NIH. September 15, 1998 to June 30, 2002. Total funds $786,201 RO1 NRO4527-01A1
- Study on cholera and *Vibrio cholerae* in the environment. Funded by J. Epstein Foundation. January 1, 2001 to December 31, 2002. Total funding $100,000.
- Study on cholera and *Vibrio cholerae* in the environment. Funded by ÇOUG Foundation. June 14, 2001 to June 13, 2002. Total funding $50,000.

Check List

After completing the protocol, please check that the following selected items have been included.



1. Face Sheet Included ✓
2. Approval of the Division Director on Face Sheet ✓
3. Certification and Signature of PI on Face Sheet, #9 and #10 ✓
4. Table on Contents ✓
5. Project Summary ✓
6. Literature Cited ✓
7. Biography of Investigators ✓
8. Ethical Assurance NA
9. Consent Forms NA
10. Detailed Budget ✓

Protocol "Environmental persistence of *Vibrio cholerae*" (protocol # 2001 - 018)

PI: Md. Sirajul Islam (local)

The protocol will study the environmental factors which determine the gene expression of *Vibrio cholerae* under changing environmental conditions. It is expected that this research will contribute to better understanding of the timing and severity of cholera outbreaks and long term survival of *Vibrio cholerae* in the environment.

The investigators are well experienced in conducting these types of environmental studies. They already conducted other environmental studies relating to ecology of cholera at Matlab.

I have the following comments:

1. The investigators could provide some background information of the studies already conducted at different ponds of Matlab and Chandpur areas (salinity, pH, water quality, algae concentration, persistence of *Vibrio cholerae* in different seasons etc).

2. Project summary should contain a brief statement of the problem and study sites.

3. Figure 4 needs more clarification (x axis labels should be clearly marked). All figures should be sequentially numbered.

4. What is the criteria of selection of one pond at Matlab and the one at Chandpur area?

5. Briefly mention the methods of measurements of chlorophyll, orthophosphate, dissolved organic carbon and total solids in water collected from ponds.

6. The study will involve a lot of statistical methods for the analysis of data. I did not find any statistician/epidemiologist in the investigators' list.

7. Other costs (US $ 73,894) need to be specified.

8. I have not found comments from external reviewers.

Thanks

Dr. K. Zaman

Protocol "Environmental persistence of *Vibrio cholerae*" (protocol # 2001 - 018)

PI: Md. Sirajul Islam (local)

The protocol will study the environmental factors which determine the gene expression of *Vibrio cholerae* under changing environmental conditions. It is expected that this research will contribute to better understanding of the timing and severity of cholera outbreaks and long term survival of *Vibrio cholerae* in the environment.

The investigators are well experienced in conducting these types of environmental studies. They already conducted other environmental studies relating to ecology of cholera at Matlab.

I have the following comments:

1. The investigators could provide some background information of the studies already conducted at different ponds of Matlab and Chandpur areas (salinity, pH, water quality, algae concentration, persistence of *Vibrio cholerae* in different seasons etc).

2. Project summary should contain a brief statement of the problem and study sites.

3. Figure 4 needs more clarification (x axis labels should be clearly marked). All figures should be sequentially numbered.

4. What is the criteria of selection of one pond at Matlab and the one at Chandpur area?

5. Briefly mention the methods of measurements of chlorophyll, orthophosphate, dissolved organic carbon and total solids in water collected from ponds.

6. The study will involve a lot of statistical methods for the analysis of data. I did not find any statistician/epidemiologist in the investigators' list.

7. Other costs (US $ 73,894) need to be specified.

8. I have not found comments from external reviewers.

Thanks

Protocol "Environmental persistence of *Vibrio cholerae*" (protocol # 2001 - 018)

PI: Md. Sirajul Islam (local)

The protocol will study the environmental factors which determine the gene expression of *Vibrio cholerae* under changing environmental conditions. It is expected that this research will contribute to better understanding of the timing and severity of cholera outbreaks and long term survival of *Vibrio cholerae* in the environment.

The investigators are well experienced in conducting these types of environmental studies. They already conducted other environmental studies relating to ecology of cholera at Matlab.

I have the following comments:

1. The investigators could provide some background information of the studies already conducted at different ponds of Matlab and Chandpur areas (salinity, pH, water quality, algae concentration, persistence of *Vibrio cholerae* in different seasons etc).

2. Project summary should contain a brief statement of the problem and study sites.

3. Figure 4 needs more clarification (x axis labels should be clearly marked). All figures should be sequentially numbered.

4. What is the criteria of selection of one pond at Matlab and the one at Chandpur area?

5. Briefly mention the methods of measurements of chlorophyll, orthophosphate, dissolved organic carbon and total solids in water collected from ponds.

6. The study will involve a lot of statistical methods for the analysis of data. I did not find any statistician/epidemiologist in the investigators' list.

7. Other costs (US $ 73,894) need to be specified.

8. I have not found comments from external reviewers.

Thanks