Journal of Industrial Microbiology & Biotechnology (2000) 24, 334–338

www.nature.com/jim

Biofilms and biodiversity: an interactive exploration of aquatic microbial biotechnology and ecology

JA Frederick[1], D Jacobs[2] and WR Jones[3]

[1]*Maryland Sea Grant Extension Program, Columbus Center, 701 E Pratt St, Baltimore, MD 21202;* [2]*Maryland Sea Grant, 0112 Skinner Hall, College Park, MD 20742;* [3]*Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 E Pratt St, Baltimore, MD 21202, USA*

The study of biofilms provides a unique educational opportunity to examine ecosystems, biodiversity and applications of environmental biotechnology. There are many variables that could be studied for measuring the interactions between bacterial biofilms and invertebrate biodiversity as a method for assessing the health of aquatic ecosystems. These interactions also lend themselves to an easily replicated model system which can be used to reach a wide audience with an educational opportunity for students as well as a professional development opportunity for teachers. At the foundation of the research are invaluable basic microbiology skills: strain collection, isolation, cultivation and characterization. Through the additional process of characterizing, identifying and enumerating invertebrate organisms that attach to bacterial biofilms in aquatic ecosystems, there evolved a multidisciplinary class laboratory activity that has found broad application. This activity is captivating not only to undergraduate microbiology students but to middle and high school students and their teachers. The demand for information about the activity has led to the development of a truly interactive web-based lesson, which in turn has resulted in additional inquiries and further refinement of the lesson as an undergraduate independent research course. Both of these are freely accessible on the web, with growing international participation and data exchange. *Journal of Industrial Microbiology & Biotechnology* (2000) **24,** 334–338.

Keywords: biofilms; biodiversity; marine microbiology; microbial ecology

Introduction

The University of Maryland Biotechnology Institute's Center of Marine Biotechnology (www.umbi.umd.edu) and Maryland Sea Grant (www.mdsg.umd.edu) have a unique collaboration which serves to bring scientists and aspects of their ongoing research to visiting school groups, using fully equipped research laboratories designated for student use and staffed with experienced educators. In the example we describe here, microbial biofilm research has been transformed into a hands-on and on-line opportunity for teachers and students locally, nationally, and internationally. The focus is on the study of bacterial biofilms and the aquatic organisms that colonize them [1,3]. Microbial biofilms on surfaces are of fundamental importance in aquatic ecosystems. They constitute a specific habitat totally different from the bulk water environment and consequently there are biological processes that are unique to biofilms. Biofilms are of interest for their negative influence on artificial surfaces in aquatic environments, such as increased drag on ship hulls due to the pivotal role of biofilms in the process of macrofouling as well as microbially-induced blistering and corrosion of coated metal surfaces. Biofilms are also of interest because they may, in the future, be used to counter or control these processes as well as to affect other processes such as bioremediation of environmental contaminants. These aspects of microbial ecology and biotechnology research serve as the basis of this educational program. Data collection, dissemination and independent research are facilitated through an interactive web page that expands biotechnology education to include the study of aquatic biofilm samples and their role in environmental biotechnology.

These miniature ecosystems are investigated by retrieving transparent plexiglass disks that have been previously submerged at numerous sampling sites for varying periods of time, ranging from 1 to 4 weeks or more (Figure 1). Over a short time, a bacterial biofilm grows on these disks, which will also then serve as home to barnacles, oyster larvae and many other marine organisms, creating a microcosm of highly animated biological activity which is fascinating to observe, even under low-power magnification (Figure 2). The effects of numerous physical water quality parameters on this process provide an ideal opportunity for scientific endeavor and discovery. While microbiology is fundamental to the process, other areas of research include biodiversity, marine ecology, and environmental biotechnology, as well as cellular and molecular biology.

Due to the substantial positive impact this opportunity has provided, it is now being regarded as a model which can be applied to many other research projects that are relevant to the general public and the goals set forth by local and national education communities. In addition, this activity has attracted an international audience and has become the focus of a collaborative research project between teachers and students in Maryland, Sweden, and Norway. Ultimately, these student researchers are contributing to the scientific database and proving themselves to be a legitimate if not invaluable source of scientific data.

Correspondence: WR Jones, Head of Educational Programs, Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 701 E Pratt St, Baltimore, MD 21202, USA. E-mail: jones@umbi.umd.edu
Received 19 April 1999; accepted 17 February 2000

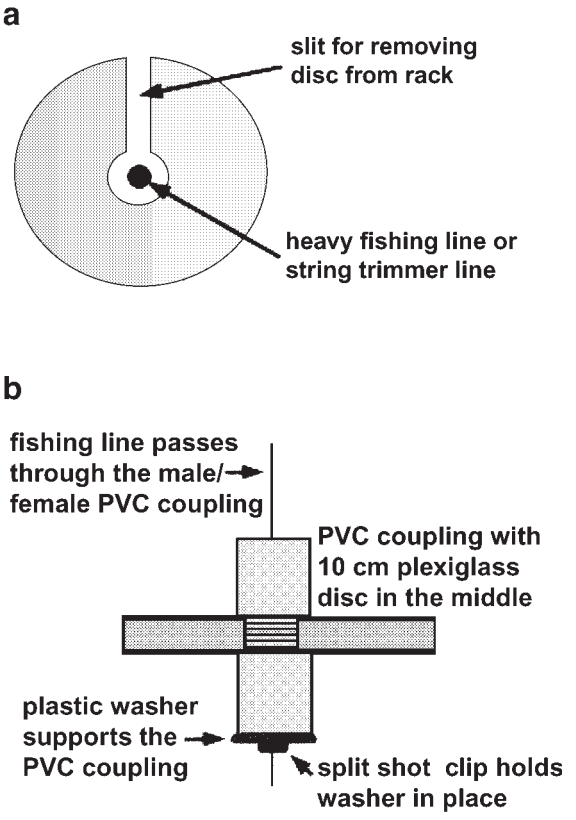

Figure 1 Basic biofouling collection rack design. (a) The design of the collection rack is simple and inexpensive to build. A hole was drilled in the center of the 10-cm plexiglass discs and a narrow slit was cut from the center to the edge. The slit allows the user to slide the disc off the fishing line and to remove an individual disc from the rack. The size of the discs can vary, but a 10-cm disc fits perfectly in a tupperware sandwich container or ziplock baggie for transport to the lab and they are easy to view under a stereoscope. (b) Illustrates how the male/female threaded PVC coupling fits through the hole in the center of the disc and is tightened until the disc is held in place between them. The plastic washer has a slightly greater diameter than the male/female PVC coupling and supports it from the bottom. The split shot is pressed on to the line just below the plastic washer and the disc is held in place at a predetermined location. There are many interesting experiments that can evolve by varying the depth of the discs and the distance the dics are placed from one another along the line.

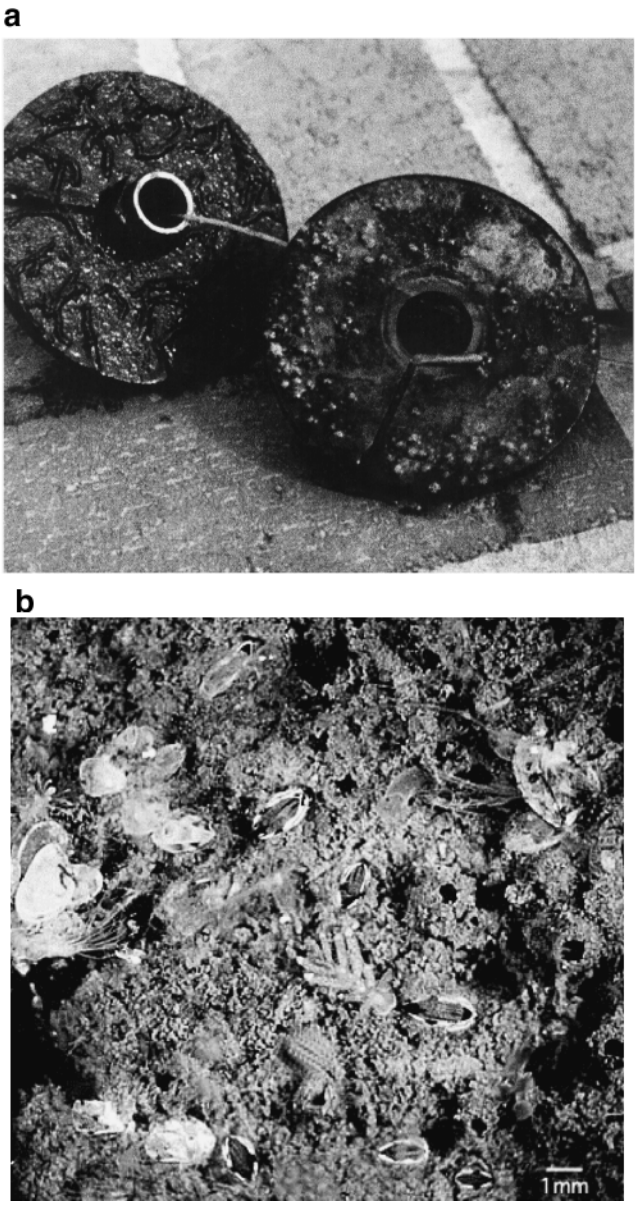

Figure 2 Biofilm disk with biofouling. (a) Some crusty discs after 4 weeks in the Baltimore Inner Harbor. The disc on the left has an abundance of whip mud worms and the disc on the right has a number of small barnacles. (b) A disk with high biodiversity under low magnification.

With the fast pace and growing importance of technology-oriented fields such as science and biotechnology, highly-trained scientists/educators must help middle and high school teachers remain current in the field and help students become and stay actively engaged with science throughout their lives. To this end, we have begun sharing successful SciTech education programs on a national and international level by developing the hands-on 'Biofilms and Biodiversity' laboratory investigation as an interactive web-based lesson (www.mdsg.umd.edu/Education/biofilms/intro.htm). This lesson enables a participant to learn about the research by manipulating data, applying math skills, identifying biofilm organisms, and developing an individual research project. The approach of providing all the necessary information on the web, and interactive training, exploits the true utility of this rapidly developing virtual venue. The extensive web-based lesson is designed for both teachers and students and can be used in multiple ways: (1) interactively in class or as a follow-on activity to a hands-on laboratory investigation (eg, some teachers have used two classroom periods having students pursue this web page, effectively integrating technology into the classroom); (2) independent from any field experiment/laboratory activity (eg, virtual biofilm samples are used as the basis of study); and (3) as an initiation point for independent research and/or a science project.

As an interactive learning tool, this lesson makes learning science engaging, productive and fulfilling without the usual sacrifice of rigor and content. It also allows students from all over this country and others, even those not in close proximity to the ocean, to learn about microbiology and biodiversity without participating in a laboratory experiment. Alternatively, because the concepts of micro-

biology and biodiversity apply to fresh water and terrestrial environments as well, teachers and students who first learn about microbiology and biodiversity on their computer may wish to adapt the lesson for laboratory investigations based on local field conditions. In addition, biotechnology is one of science's most dynamic fields and one that is little understood by middle school and high school teachers. Applications of biotechnology in the real world and the use of web-based technology in a scientific context make the topic especially engaging for teachers and students. The combination of biotechnology and marine science in particular has proven to be so appealing that many students, including those at-risk [4], have demonstrated renewed interest and enthusiasm in both science and math after participating in this program (personal communication, Bryan Stoll, Woodlawn Senior High School, Baltimore, MD). In addition, we have documented that through the adaptation of authentic research and the opportunity for hands-on experience, comprehension and retention of the information is increased.

Some schools do not have the resources to support traditional microbiology education, so the lessons provided to teachers during this program are designed to be easily replicable in the classroom using safe, inexpensive, everyday materials. Through our program we are also able to provide alternative processes when availability of equipment or supplies is an issue. Additionally, we provide guidance on how to seek funding for educational program development in the classroom, as needed. This may involve acquiring a relatively inexpensive stereoscope for the collection of data and/or digital imaging equipment for the capture and exchange of images.

Materials and methods

Research internship

Internship opportunities in the summer are the first step toward providing teachers with extensive first-hand experience in the laboratory and are fundamental to the introduction of new concepts and laboratory activities in the classroom. Although this serves a limited number of teachers at the outset, expansion by mentoring between teachers allows many of the concepts and activities to reach a broader audience.

The purpose of the initial teachers' summer laboratory internship is to characterize environmental isolates of biofilm-forming bacteria for differential effects on colonization by invertebrates in the marine environment. An *in situ* approach is used for its relevance and direct application to understanding the phenomenon of invertebrate colonization, or macrofouling, and how to control it. In addition, this approach provides an opportunity to describe interactions between individual bacterial strains and individual invertebrate species that provide model systems through which to study the process of macrofouling at the cellular and molecular level. This approach involves characterizing environmental isolates of biofilm-forming bacteria, culturing them individually on plexiglass discs, deploying them on racks in the estuarine ecosystem from which they were isolated, retrieving them over time, and comparing their invertebrate populations (Figure 3).

Class laboratory activity

As an outgrowth of the research, educational extension activities were developed and directed at enhancing learning about marine biotechnology for a broad audience. The first of these is an on-going laboratory activity offered in the SciTech education program and entitled Biofilms and Biodiversity. This half-day laboratory activity offers the opportunity for students and teachers in grades 6 through 12 to experience the process of collecting samples of biofilms on discs from the Baltimore Inner Harbor and analyzing them in the SciTech lab. The stereoscopic analysis of the biofilm community is the 'hook' that excites students and teachers, facilitating a deeper understanding of how and why microbial biofilms and biodiversity are studied. The development of the laboratory activity was simple and required very basic equipment which makes it universally applicable to the development of follow-on activities and projects in the classroom.

Interactive Web Page

In an effort to increase the appeal of the biofilms and biodiversity lesson, a collaboration with Maryland Sea Grant led to development of an extensive interactive web-based lesson hosted on the Maryland Sea Grant web page (www.mdsg.umd.edu/Education/biofilms/intro.htm). A lesson was created that allows the input of real-time data and instant feedback of information including the analysis of various biodiversity indices calculated from data collected at different locations. The web lesson features JavaScript programming that enables users to learn how biodiversity analysis is performed, practice on virtual biofilm samples, and construct their own project and analytical data tables. Highlights include:

- An *Introduction* page to the broad topic of microbial biofilms.
- A *Featured Creatures* page that displays and describes aquatic organisms commonly found in biofilm communities in the Inner Harbor, Baltimore, MD.
- A *Tutorial* page on the use of biotic indices to analyze biofilm populations.
- A *Virtual Samples* page of two biofilm communities from Baltimore's Inner Harbor where users can perform simulated data analysis.
- A series of *Experimental Design* pages that help the user design and implement an appropriate biofilm field study.
- A *Teacher Resource* page that describes the connection of national and state education goals and their relationship to the biofilms and biodiversity lession.

Ultimately, the intent of the web lesson is to increase awareness of proper use of the scientific method for experimental design and implementation.

International collaboration

The Virtual University Education (VIRTUE) program is a collaboration between the University of Maryland (US), the University in Bergen (Norway) and Göteborg University (Sweden) funded by the Wallenberg Foundation (www.umbi.umd.edu/virtue). It is comprised of research projects, curricula development for graduate and undergrad-

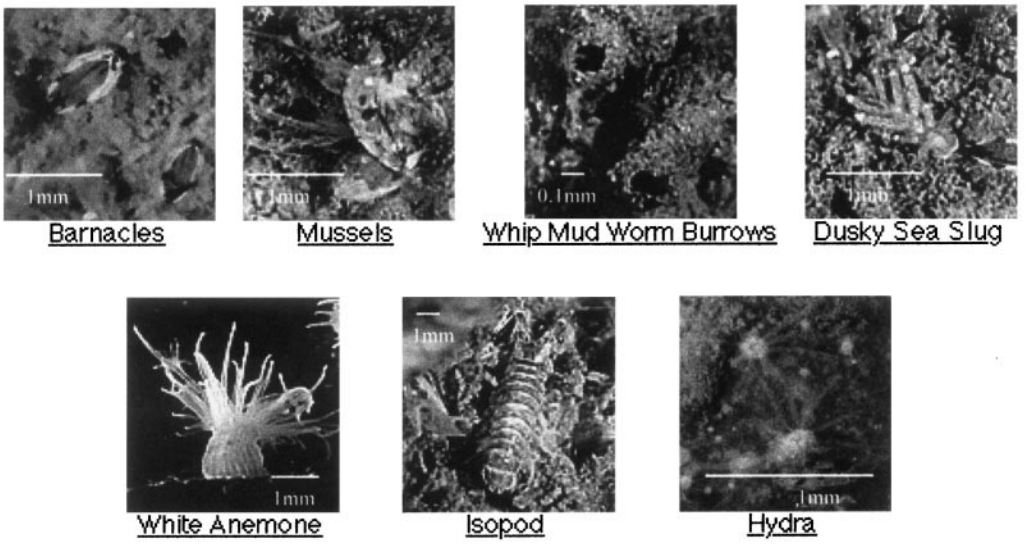

Figure 3 Featured creatures.

uate students, continuing education of teachers and classroom programs for students.

The intriguing nature of the biofilms and biodiversity lab and web-based lesson has attracted the attention of the Public Outreach Committee of the VIRTUE program. This committee is dedicated to the translation of the VIRTUE research projects into the public realm via a variety of methods, including videoconferencing. One goal of the VIRTUE Public Outreach Committee is the development of a science project that could be synchronously implemented in Maryland, Sweden, and Norway by high school teachers. The committee decided that the biofilms and biodiversity lesson could be a catalyst for one such project and it was scheduled for start-up in 1999.

Independent research course

Through involvement in Web Initiatives in Teaching (WIT; www.umuc.edu/ide/wit/), we refined the Biofilms and Biodiversity lesson in order to provide a formal, web-based course designed for undergraduate students who would like to participate in a faculty-mentored independent research opportunity (www.mdsg.umd.edu/wit/). For example, with access to readily available laboratory equipment and a lotic or tidal aquatic ecosystem, a student can enroll locally in the web-based course in order to design and carry out a structured research project incorporating individualized scientific inquiry.

Results

Research internship

The initial results from characterizing biofilm-forming bacterial isolates included those cases in which: (1) there were no observable differences in the characteristics and rate of succession to macrofouling; (2) those in which differences appear but do not persist; and (3) those in which differences in types and numbers of invertebrates that appear and persist can be correlated with differences between species of biofilm-forming bacteria. One bacterial strain, a Gram-positive isolate that appears by membrane fatty acid analysis to be an as yet unnamed *Bacillus* species, was shown by Simpson's index calculations (a standard index for measuring biodiversity) to give rise to a decrease in biodiversity of the invertebrate community that colonizes its surface [7]. Over a 19-day test period this isolate showed a decline in biodiversity (as measured by the reciprocal of Simpson's Index) at a rate of −0.10 units per day while its control (sterile plexiglass disks) showed an increase in biodiversity at a rate of 0.14 units per day during the same test period. Three other isolates (*Aeromonas sobria*, *Shewanella putrefaciens*, and *Hyphomonas* M-3) showed increases in biodiversity at rates of 0.34 units per day, 0.23 units per day, and 0.07 units per day, respectively. The *Bacillus* sp and several other bacterial strains of interest are being identified by 16S rNA sequencing while additional isolates continue to be screened. We have identified a subset of sentinel invertebrate species that routinely colonize the biofilm surfaces so the potential exists to identify model interactions between individual bacterial strains and particular invertebrate species. These interactions will then be characterized at the molecular level in order to further our understanding of the roles of signal molecules, surface proteins and other factors [2].

Class laboratory activity

The Biofilm and Biodiversity half-day lab for students and teachers has significantly increased awareness of the microbial nature of biofilms and their importance in establishing the macro-fouling community. With the involvement of graduate students and post-doctoral fellows in the SciTech education program, middle school and high school students and teachers are able to get 'hands-on' experience and interact directly with research staff. The enthusiasm generated by participation in this laboratory activity illustrates the need for development of additional activities that enhance experiences in microbiology.

Interactive web page

The web page for the Biofilm and Biodiversity activity is visited on a regular basis and has been adopted by middle

schools, high schools, undergraduate students and other groups. The activity has been described in various media as well, giving rise to numerous inquiries by phone and e-mail [5,6]. In addition, it has also been incorporated into the science research curriculum in a local secondary school system. Those who we have subsequently referred to the web page report that they have found it self-explanatory and were able to use it right away to begin collecting data.

International collaboration

During the spring of 1999, teachers from the three participating countries (US, Norway and Sweden) met to discuss a common marine science project, suitable for students at the upper secondary and high school levels. The Biofilms and Biodiversity activity was chosen and discs are being deployed in the water at different locations while environmental factors are measured. As the discs are brought back to the laboratory the biofilms and biodiversity are being studied as previously described.

The first step has been to educate teachers so they can take their new knowledge to the classroom. This was done during the spring of 1999. In autumn 1999 students in all three countries started deploying the discs then reporting and discussing their findings with each other by means of the Internet, e-mail and videoconferences. With the discs as the common tool students are investigating what's growing on the discs and why, what differences occur between different locations, and how these differences correlate with differences in water quality.

Independent research course

The Biofilm and Biodiversity independent research course has been fully developed as part of the institution-wide Web Initiatives in Teaching program (www.umuc.edu/ide/wit/). As posted on the web, it has been accepted for credit at other universities (www/mdsg.umd.edu/wit/). The course is being used as an example for web-based instruction that can be offered 'anytime and anywhere'. This is largely because it involves ongoing research of unspecified duration, serving to avoid the boundaries of time and location.

Discussion

Microbial biofilm research provides the foundation for a hands-on and on-line opportunity for teachers and students locally, nationally, and internationally. The focus is on study of bacterial biofilms and the aquatic invertebrates that colonize them. This provides a highly adaptable, easily disseminated, multidisciplinary model system for the study of microbiology, biodiversity, environmental biotechnology and other topics. The program is now being regarded as a model which can be applied to many other research projects of interest that relate to the general public and the goals set forth by local and national education communities. In addition, now that this collaboration has attracted an international audience it has become the focus of a collaborative research project between teachers and students in US, Sweden, and Norway. This educational program has also evolved to serve as a credible source of scientific data. This has begun through publication of a peer-reviewed abstract and presentation at a national meeting by a research intern [7]. In addition, Norway's national water program (the Norwegian Vannprogrammet) has, in consideration of the validity of the data, agreed to include the data in their official web database.

Intriguing aspects of microbiology have served as a catalyst in creating a program that has become self-propelled and self-perpetuating, through a cycle in which laboratory research internships feed directly into classroom activities, with international participation and dissemination via the web giving rise to more extensive laboratory research internship opportunities.

Acknowledgements

We acknowledge our education specialists Janine Azzouz and Jeff Morgen for their tireless commitment, Rober Baier for essential discussions and the Wallenburg Foundation for support of the VIRTUE program.

References

1 Costerton JW. 1995. Overview of microbial biofilms. J Ind Microbiol 15: 137–140.
2 Davies DG, MR Parsek, JP Pearson, BH Iglewski, JW Costerton and EP Greenberg. 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 280: 295–298.
3 James GA, L Beaudette and JW Costerton. 1995. Interspecies bacterial interactions in biofilms. J Ind Microbiol 15: 257–262.
4 Lieberman GA and LL Hoody. 1998. Closing the achievement gap: using the environment as an integrating context for learning. State Educational and Environment Roundtable. Science Wizards: Poway, CA.
5 Maryland Sea Grant Schools Network News. 1999. Maryland Sea Grant on the World Wide Web 1: 1–3.
6 NSTA Reports. 1999. Marine Science Project on Fouling Organisms. Hotline, pp 18–19.
7 Stoll BG, J Tinsely, JA Frederick and WR Jones. 1999. Measuring effects of bacterial biofilms on biodiversity in aquatic ecosystems. Abs Annu Meet Am Soc Microbiol, p 665.