Mentoring students conducting independent research on antibiotic production by marine Actinomycetes

Acompañamiento a estudiantes que realicen investigaciones independientes sobre la producción de antibióticos por Actinomicetos marinos

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Abstract

This paper presents research on antibiotic production by marine actinomycetes conducted by a high school student and a community college student under the supervision of a college faculty mentor. The benefits of student involvement in conducting individualized research projects are presented as a major component of this paper.

Key words: *actinomycetes, antibiotic production, mentoring, student research*

Resumen

Este trabajo presenta una investigación sobre la producción antibiótica por actinomicetos marinos, dirigida por estudiantes de la escuela secundaria y de universidad bajo la guía de un docente de universidad. Se presentan los diferentes beneficios educativos para los estudiantes que llevan a cabo proyectos de investigación individualizados.

Palabras clave*: actinomicetos, producción antibiótica, investigación de estudiantes.*

INTRODUCTION

In order to emphasize science as a process and to promote students overcoming possible uncertainties or fear of science, a wide variety of new courses, programs, and opportunities for students to experience science have been developed in recent years (WUBBELS and GIRGUS, 1996). So that science can be pursued well beyond the more traditional lecture/laboratory course offerings, such programs are developing at all educational levels, elementary, high school, and colleges. The primary aim of many of these newer programs is to expose students, particularly those traditionally underrepresented in the sciences, to the process of science that can be conducted by students at different educational levels (CASE and WARNER, 2000; Greengrove and SECORD, 2003). These efforts have promoted greater scientific confidence, direct scientific involvement, and increased student numbers in scientific endeavors in accordance with the recommendations set for by *National Science Education Standards* (NAS 1995).

Through a series of grants, the author/faculty mentor began a long term research project at Kingsborough Community College (KCC) on the isolation of antibiotic-producing marine actinomycetes from Jamaica Bay. Jamaica Bay is almost entirely in the boroughs of Brooklyn and Queens in New York City and is separated from the Atlantic Ocean by the Rockaway Peninsula. JOHN F. KENNEDY International Airport extends into Jamaica Bay and this bay is part of Gateway National Recreation Area, one of the largest urban national parks. Using the vessels available at the college, the author collected marine sediments from four samples sites and isolated actinomycetes from these sediments. This initial work led to the development of several other research projects, all dealing with antibiotic-producing marine actinomycetes (Table 1). Another grant, the Louis B. Stokes Alliance for Minority Participation (LSAMP) gave students the opportunity to conduct research with a faculty mentor. This national project, funded by the National Science Foundation was awarded to CUNY and provided funding for students to work with volunteer faculty mentors. Students interested in participating in LSAMP were directed to the author. The research presented in this paper was conducted by one community college student working with a faculty mentor over the year he participated in the LSAMP project. In addition, one of the local high schools in Brooklyn, Midwood High School, was seeking volunteer college faculty to guide and involve students on research projects, such as the highly competitive Westinghouse project, Intel, and other science competitions. The additional data presented in the paper were a result of research conducted by a volunteer high school student working with the college faculty mentor. The effects on the students and their future academic endeavors will also be described in this paper.

Table 1
Additional Research Projects Conducted on Marine Actinomycetes by Students with a Faculty Mentor

- Determination of chemical and physical properties of marine water and sediment samples.
- Correlation of environmental parameters to the presence of antibiotic producing marine actinomycetes.
- Heavy metal resistance in antibiotic producing versus nonproducing marine actinomycetes.
- Enhancement of anti-acid-fast antibiotic production by marine actinomycetes.
- Comparison of antibiotic production by actinomycetes isolated from marine sediment in Jamaica Bay and terrestrial soil samples.

BACKGROUND

The advantage to the producer organism in synthesizing such compounds is not yet known. Research is continuing in the field of secondary metabolism and antibiotic production to determine Actinomycetes are bacteria commonly found in soil that are well known for their ability to produce antibiotics. Antibiotics, as well as pigments and odors, are classified as secondary metabolites. These compounds are produced by organisms at the end of their life. Howeverthe precise conditions required to consistently acquire antibiotic production. There are many factors, such as temperature, aeration, age of culture, media composition and plasmid based gene regulation, known to affect antibiotic production.

Due to the development of antibiotic resistance, there is a growing need and interest to continually discover and seek new antibiotics. To accomplish this, scientists are isolating organisms, such as algae, jellyfish, fungi and bacteria in the hopes of isolating more effective active compounds (FENICAL, 1997; KONIG and WRIGHT, 1996). Marine actinomycetes, as contrasted with the commonly studied terrestrial actinomycetes are also being isolated. Prior studies have demonstrated actinomycetes isolated from marine sediments are capable of producing antibiotics which might be novel and potentially useful compounds (PISANO, *et al.* 1989).

MATERIALS AND METHODS

Sediment Sample Collection

Sediment sampling was conducted by the faculty mentor using one of KCC's vessels and crew at depths of 15-25 feet with a benthic box. Samples were taken from 4 sites in Jamaica Bay. When returning to the lab, all sediment samples were stored at 4°C in sterile specimen cups until they were used to isolate actinomycetes.

Isolation of Actinomycetes from Marine Sediments

Dilutions (10^{-1} -10^{-4}) of one gram of sediment in sterile water were prepared and plated on appropriate media, actinomycete isolation medium, chitin agar, and starch-casein agar to isolate actinomycetes. (The recipes for the media are listed in Table 2). All media were supplemented with 15µg/ml of nystatin and 15µg/ml of cycloheximide to inhibit fungal growth. These plates were incubated for four weeks at 28°C. After the four weeks of incubation, the plates were observed and colonies were purified on glucose peptone agar plates that had the characteristic powdery, grey, brown appearance of actinomycetes. All plates were incubated at 28°C for one

additional week. In the fifth week, these purified colonies were examined macroscopically and microscopically. Based on these observations, colonies preliminarily identified as actinomycetes were then grown in two types of fermentation broths, daunomycin and yeast extract (YE) broth, for 72, 96 and 120 hours in a rotary shaker set at 250 revolutions per minute in order to test them for antibiotic production.

Table 2
Recipes for Media Used for Actinomycete Isolation and Testing for Antibiotic Production

Actinomycete Isolation	AIM	22.0 g
Medium (AIM)	Glycerol	5 g
(Difco)	d H_2O	1000ml
Chitin Agar	Agar	20.0 g
	Chitin	4.0 g
	K_2HPO_4	0.7 g
	$MgSO4.7H_2O$	0.5 g
	KH_2PO_4	0.3 g
	$FeSO_4.7H_2O$	0.01 g
	$MnCl_2.\ 4H_2O$	0.0001 g
	$ZnSO_4.7H_2O$	0.0001 g
	d H2O	1000 ml
Starch Casein Agar	Agar	18.0 g
	Starch	10.0 g
	Casein	1.0 g
	d H_2O	1000 ml
Glucose Peptone Agar	Glucose	50.0 g
	Agar	18.0 g
	Peptone	15.0 g
	d H_2O	1000 ml
Yeast Extract (YE) Broth	Malt extract	10.0 g
	Yeast extract	4.0 g
	Glucose	4.0 g
	d H_2O	1000 ml
Glucose Peptone Broth	Glucose	50.0 g
	Peptone	15.0 g
	d H_2O	1000 ml

Testing Marine Actinomycetes For Antibiotic Production

Actinomycetes were tested for antibiotic production against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Serratia marcescens*), acid-fast bacteria (*Mycobacterium smegmatis*), yeast (*Candida albicans*), and filamentous fungi (*Aspergillus niger*) by a standard agar diffusion assay similar to that carried out for Kirby-Bauer testing of antibiotics. Glucose peptone agar plates were swabbed with the test organisms. Then, sterile paper disks saturated with the 72 hour old actinomycete culture broths were placed on the seeded plates. Bacterial plates were incubated at 37°C for 24-48 hours; fungal and acid-fast bacterial plates were incubated at 28°C for 72-96 hours. After incubation, zones of inhibition were measured in millimeters. This procedure was also repeated for the cultures grown for 96 and 120 hours.

Optimizing Antibiotic Production by Altering Media Composition

In previous studies, dilution of nutrients has been shown to enhance antibiotic production. Also, for cultures isolated from marine environments, supplementation of the fermentation media with NaCl has also shown to enhance antibiotic production (Okami, 1982). Based on these studies, one student tested the isolated antibiotic producing actinomycetes showing activity against Gram-positive bacteria while the other student tested the isolated actinomycetes displaying antifungal activity in the following five fermentation media: yeast extract, half-strength yeast extract, yeast extracted supplemented with 3% NaCl, daunomycin, and daunomycin supplemented with 3% NaCl. The method for testing antibiotic production was the same as previously described. Each student performed three trials for each culture.

RESULTS AND DISCUSSION

Actinomycete Isolation

Using three different isolation media, starch-casein, actinomycete isolation medium and chitin agar, 106 actinomycetes were isolated. Fifty- three (50%) of these were isolated using starch-casein agar; 29 (27%) and 24 (23%) were isolated using chitin and actinomycete isolation medium respectively.

Based on the initial fermentation data collected, 44 antibiotic producing cultures were found. Some of these cultures were believed to produce more than one antibiotic based the variety of bacteria and fungi whose growth they could inhibit. Twenty-three actinomycetes were found to inhibit the growth of Gram-positive bacteria. An additional 23 actinomycetes were found to have antifungal activity. Twenty actinomycetes inhibited the growth of acid-fast bacteria. Finally, no actinomycetes were isolated that inhibited the growth of Gram-negative bacteria. For further studies of enhancing of antibiotic production, one student studied the 23 actinomycetes that demonstrated activity against Gram-positive bacteria; the other student studied the 23 actinomycetes that demonstrated activity against fungi.

Enhancement of Antibacterial Antibiotic Production by Altering Fermentation Media Composition

When the student tested the 23 actinomycetes displaying activity against Gram-positive bacteria in five different fermentation media, 17 isolates displayed consistent activity with a zone size less than 9 mm. Upon consultation with the mentor, the student repeated these experiments. After three trials, the student and mentor reviewed the results and decided to study 6 isolates that consistently displayed activity against Gram-positive bacteria with a zone size greater than 10 mm.

In the studies of the 6 actinomycete isolates, daunomycin fermentation medium was found to produce the greatest antibiotic activity for 5 of the 6 (83%) isolates as compared to all other fermentation media used in this study (daunomycin supplemented with 3% NaCl, YE, half-strength YE, and YE supplemented with 3% NaCl). Using YE broth, 3 of the 6 cultures (50%) showed increased antibiotic activity when the medium was supplemented with 3% NaCl. Half-strength YE displayed almost identical zone sizes as full strength YE.

Enhancement of Antifungal Antibiotic Production by Altering Fermentation Media Composition

For the 23 actinomycete isolates demonstrating antifungal activity, the student performed fermentation studies in 5 media. Fifteen cultures demonstrated consistent antibiotic production and these were further studied. Cultures grown in daunomycin were found to generate greater antibiotic production than those cultures grown in YE broth. After three separate trials, 8 of the 15 (53.3%) displayed maximum antibiotic production in daunomycin supplemented with 3% NaCl. Maximum antifungal activity was seen in daunomycin for 5 of the 15 isolates (33.3%). Although YE broth was not as successful a fermentation medium as daunomycin, 2 of the 15 isolates (13.3%) displayed maximum antifungal activity in YE supplemented with 3% NaCl.

Benefits to Students from Conducting Research Projects with a Faculty Mentor

The previously described results gathered by the two students agree with prior studies reporting daunomycin is a useful fermentation medium to support antibiotic production. In addition, supplementation of fermentation media with salt enhances antibiotic production from many actinomycetes isolated from salt containing environments. Research on actinomycete antibiotic production has advanced into the areas of DNA analysis to assist in the isolation of antibiotic producing microbes and the genes involved in this process. While the research reported in this paper is not as advanced as these studies, these projects were selected for the two students so they would have a successful research experience in which they were able to conduct experiments and collect data as suggested by Shellito, *et al.* (2001). Also, the equipment and facilities available at community colleges are not as extensive as the lab space, equipment, funding, and technical staff of colleges with strong research commitments. In fact, many undergraduate teaching colleges do not strongly invest in research activities (Foos, 1999).

Both students learned basic microbiological techniques prior to the start of their research project including media preparation and sterilization, aseptic transfer and Kirby Bauer sensitivity testing by the mentor. Detailed safety procedures were also explained including proper handling and disposal of microbes and chemicals. In addition, when the students conducted their experiments, the faculty mentor was in the lab as a safety precaution but the students performed independent and self-directed work. While none of these students had ever taken a microbiology course, they effectively performed laboratory techniques for the culturing and maintenance of microbes through a learning experience provided beyond traditional lecture/lab format.

During the year each student conducted their research, they met regularly with their mentor to set a course of action. Work for each week was planned, and it was the student's responsibility to make the necessary materials, including agar plates, fermentation media, sterilizing paper disks, and to come to the laboratory each day to carry out their work. The experiments described were conducted during the months of June and July and during the fall and spring semesters and required the students to work in the laboratory at least 4 days a week for one to two hours. Occasionally the students worked together or assisted one another in preparation of media if one student could not come to the lab due to other commitments. Upon completion of the students' weekly experiments, they met with the faculty mentor to review results and discuss the significance of the data they gathered. If any results were determined to be questionable, the students repeated their experiments. Students participated in this decision based on their reading of articles given to them by the faculty mentor and ones they also collected. Although at times a long and frustrating process, the students began to understand the importance of consistent data collection, the arduous efforts frequently required in isolating and developing novel antibiotics, and that studies on antibiotic production are far more difficult than they had possibly imagined. By conducting the experiments previously described, the students began to appreciate the content, process, and nature of science.

In addition, the students began to recognize some of the dimensions of the field of microbiology including ecology, soil and water chemistry, and marine science. Through the examination of their results, the students developed a capacity to think critically, to ask more questions, and to make an important discovery about experiments. That is, research processes and resulting data often lead to simply more questions requiring further experimentation rather than definitive conclusions. The overall effect was that students, by carrying out bona fide scientific practices, experienced the nature and joy of science in the best traditions of investigative work.

Scientists report their findings in presentations at meetings, journal articles, and written reports. In keeping with such formats, the students were also expected to write one progress report after each semester they conducted lab work. Both students reported initial uncertainty in their capacity to convey, explain and interpret their results, but understood it to be a valuable and beneficial educational experience. In total, each student submitted three written reports during the course of their research experience. These reports allowed students to reflect on their work, rather than constantly performing one lab experiment after another, improve writing skills, and learn proper terminology and format expected in presenting scientific information. As well as these written reports, the community college student presented his work on antifungal antibiotic production at one annual LSAMP meeting in New York and a national LSAMP meeting for minority student research in New Mexico. The work accomplished by the high school student on antibacterial antibiotic production was entered in the Intel Science Competition and the New York City Branch of the American Society of Microbiology (ASM) High School Science Competition. The high school student received an award from the ASM for her work.

Beyond the students coauthoring this paper, there were other students who conducted research with this mentor. Eight other community college students participating in the LSAMP program worked on various projects related to marine actinomycetes, their ecology and biochemistry (Table 1). These other students conducted experiments but did not organize their results in a written format to determine the future direction of their research. Some of these students reported they did not have the time needed to devote to repetitive laboratory work; their course load, part-time jobs, and family responsibilities were significant deterrents to their research efforts. These extenuating priorities have also been reported by students involved in research projects at other institutions (Shellito, *et al.* 2001). The students could not continue working as LSAMP research scholars unless they completed the thought provoking process of writing about their experimental efforts and were not awarded stipends.

Upon completion of their research experience, the LSAMP students involved in research projects in the biological and physical sciences were mailed a questionnaire by the author to assess the students' experiences conducting independent lab work and the associated activities previously described in this paper. Of the nine surveys returned, 8 students continued their education to receive a bachelor's degree or beyond. However, it is noteworthy that not all students pursued further education in the sciences. Some continued in professional programs such as physical therapy, physician's assistant, and law. Others received degrees in graphic design and illustration, and political science. The students reported conducting research gave them a better understanding of how science is conducted and a greater sense of responsibility for the work they independently conducted. Statements written by the students regarding their research experiences and future educational endeavors are listed in Table 3. In addition, by working with a mentor on an individual basis they were motivated by the direct attention they received and reported improved self-confidence in addition to a better understanding of how lab work relates to their course work (Heath Bowman and Stage, 2002).

Table 3
Statements by Students Conducting Research Regarding Their Future Education

- "Conducting research with a mentor is like working in the real business world and prepared me to continue my studies to bet a degree in graphic design and illustration."
- "Working in the lab helped me improve my problem solving skills, appreciate science and determine my career goals. I am attending physical therapy school."
- "After graduating from a community college, I got a bachelor's degree and am now in a MD/Ph.D. program at New York University."
- "I graduated with a degree in nursing, got a job, and have not, as of yet, returned to school. My experiences carrying out research helped my oral and written communication skills and my ability to work with others."
- "I became more interested in other fields of study, especially the link between economics and political science. I am working at a law firm and intend to pursue a J.D./MBA at Harvard University.

CONCLUSIONS

By actively participating in relevant independent research projects in the field of microbiology, students developed competencies in carrying out procedures and using scientific equipment and they began to view themselves as more engaged and complete individuals. The value of conducting such investigations as a researcher is evidenced at all levels of education and equally so with teachers participating in courses with inquiry-based laboratories (Johnson, 2002).

Effectively completing detailed laboratory experiments involved a wide variety of skills that were improved by the involved students. By formulating an experiment based on discussions with their mentor and reading scientific articles, the students developed better organizational skills as well as the ability to think critically about their project. Through the use of basic microbiological techniques and equipment, they acquired increased manual dexterity, and technical and observational skills. As the students conducted experiments, recorded their results and made observations, they learned about time management, and sharpened skills to recognize how to reach sound experimental conclusions. Writing reports and attending meetings to present experimental findings helped to expand the students' proficiency as communicators. It is believed students mentored by faculty grow to be creative, educated individuals capable of benefiting society (Foos, 1999).

Finally, working with students as a mentor beyond traditional classroom interactions, promotes a feeling of mutual respect between students and their college professors. For this faculty mentor, it was indeed a privilege to work with motivated, inquisitive students and through this process assist them with their academic work, career aspirations, and future lifetime goals.

ACKNOWLEDGMENT

The work presented was supported through grants funded by the Research Foundation of the City University of New York, and two grants, offered through the Louis B. Stokes Alliance for Minority Participation.

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Received 12.12.2003, approved 05.05.2004