Special Section: Innovative Laboratory Exercises–Focus on Genomic Annotation

Integrating Grant-Funded Research into the Undergraduate Biology Curriculum Using IMG-ACT

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Abstract

It has become clear in current scientific pedagogy that the emersion of students in the scientific process in terms of designing, implementing, and analyzing experiments is imperative for their education; as such, it has been our goal to model this active learning process in the classroom and laboratory in the context of a genuine scientific question. Toward this objective, the National Science Foundation funded a collaborative research grant between a primarily undergraduate institution and a research-intensive institution to study the chemotactic responses of the bacterium Pseudomonas putida F1. As part of the project, a new Bioinformatics course was developed in which undergraduates annotate relevant regions of the P. putida F1 genome using Integrated Microbial Genomes Annotation Collaboration Toolkit, a bioinformatics interface specifically developed for undergraduate programs by the Department of Energy Joint Genome Institute. Based on annotations of putative chemotaxis genes in P. putida F1 and comparative genomics studies, undergraduate students from both institutions developed functional genomics research projects that evolved from the annotations. The purpose of this study is to describe the nature of the NSF grant, the development of the Bioinformatics lecture and wet laboratory course, and how undergraduate student involvement in the project that was initiated in the classroom has served as a springboard for independent undergraduate research projects.

Keywords: Bioinformatics; functional genomics; active learning

The development of rapid genome sequencing technologies and bioinformatic tools with which to interpret and compare these genome sequences has changed the way scientists formulate and address biological questions. It has become abundantly clear that current scientific pedagogy is focused on immersing students in the scientific process to develop and nurture the analytical skills required to become a scientist. These skills include the ability to critically analyze the primary literature, to design and execute properly controlled experiments that reflect real-world scientific problems, and to analyze experimental results [1–4]. Therefore, students need to be exposed to technological advances that allow scientists to use genome sequence data to investigate problems in bona fide research experiences that reflect the influence of bioinformatic tools in current life sciences research. Educators have responded by incorporating genomics and bioinformatic analyses into their curricula, ranging from single exercises in the lecture or laboratory to the development of entire courses and/or new majors that emphasize the importance of bioinformatic analysis [5–17].

It has been our goal to model the importance of bioinformatic analysis in undergraduate education in the context of a genuine scientific question. The National Science Foundation funded a collaborative research grant (NSF Award MCB-
The Project: Characterization of Chemotactic Responses in *Pseudomonas putida* F1

Chemotaxis is the ability of motile bacteria to detect and respond to specific chemicals in the environment that they can typically use as sources of carbon and energy [19]. Enteric bacteria such as *Escherichia coli* use cell-surface receptors called methyl-accepting chemotaxis proteins (MCPs) that detect the presence of chemoattractants in their environment. Binding of the chemoattractant to the MCP sends a signal to the flagellar machinery of the cell, which allows the bacterium to swim toward the attractant of interest [20]. Alternatively, some sources of carbon and energy are sensed through the actual process of metabolism via a process called energy taxis, in which membrane-bound protein receptors recognize changes in the energy status of the cell by monitoring the redox state of the electron transport chain through flavin adenine dinucleotide electron carriers [21]. Although diverse bacteria have conserved chemotaxis signal transduction systems, soil bacteria seem to have more complex chemosensory systems than the well-studied enteric bacteria. For example, enteric bacteria such as *Escherichia coli* have only five chemoreceptor proteins (four MCPs and the MCP-like energy receptor Aer; Refs. [22] and [23]), which is likely to reflect the limited range of available carbon sources present in the ecological niche in which these organisms reside. Soil bacteria such as the pseudomonads, which are known for their broad catabolic abilities, are capable of detecting a wide range of chemoattractants [24]. In addition to various sugars, amino and organic acids, pyrimidines, and inorganic phosphate [25–28], motile soil bacteria have been shown to be attracted to aromatic compounds and other toxic pollutants, many of which are growth substrates for specific strains [29–40]. Pseudomonads also appear to have correspondingly complex arrays of chemoreceptor proteins. Based on genomic sequence analyses, *Pseudomonas aeruginosa* PA01 has 26 MCP-like genes, *Pseudomonas putida* KT2440 has 27 genes, and *Pseudomonas syringae* DC3000 has 49 genes [24]; the more recently sequenced genome of *P. putida* F1 encodes 27 putative MCPs [41].

We have been particularly interested in studying *P. putida* F1 because it has the ability to grow on aromatic hydrocarbons [42] and it is also chemotactic to these toxic chemicals [34]. To date, very few of the annotated chemoreceptors in pseudomonads have been functionally characterized. One overarching goal of our research laboratories is to develop a system-level understanding of the integration of metabolism and chemotaxis in *P. putida* F1.

The broader impacts of this project include the education and training of undergraduate students; they have been immersed in this project in a number of different ways. An undergraduate capstone course in Bioinformatics, which provides hands-on training in genome annotation and functional genetics, was developed at UST (see below). Undergraduates at both UST and UC Davis carry out research projects during the school year as well as in the summer, and selected UST undergraduates spend one summer month carrying out research at UC Davis. Undergraduates at UST and UC Davis are provided the opportunity to present results of their research at the UST Department of Biology Senior Symposium and the UC Davis Undergraduate Research Day, respectively. Selected students also present findings at local and national scientific meetings (see below).

The Course: Bioinformatics

A new Bioinformatics course (BIOL464) was developed at UST in which students actively annotate the *P. putida* F1 genome and then use that information to conduct wet laboratory research projects with this organism based on their hypotheses. BIOL464 is designed as a capstone course for biology, biochemistry, and neuroscience majors with no specific set of prerequisite courses required. The only requirement is senior status; the course is appropriate for students with a wide variety of backgrounds (e.g. completion of courses in biochemistry, microbiology, genetics, cell biology, organometallic chemistry, thermodynamics, statistics, and experimental design) that are relevant to functional genomic and bioinformatic analyses. The course size is capped at 12 students.

The 15-week course involves three, 65-minute classes and one 4-hour laboratory per week (4 credits total). Students also have access to the laboratory throughout the week to allow time to work independently. The course design, which is based entirely on the primary and secondary literature, involves an intensive 2-week introduction to basic bacteriology and metabolism in *P. putida* F1, along with an introduction to chemotaxis and the motile behavior of flagellated bacteria [31,43–45]. Over the next 11 weeks, students are introduced to bioinformatics and the IMG-ACT platform [18,46,47]. Emphasis is placed on the importance of manual curation of automated genome annotations. The students are expected to master the fundamental principles and theories that underlie the field of bioinformatics by finding, reading, and presenting primary literature results pertaining to various bioinformatics databases.
that are used to generate annotations (BLAST, CDD, T-COFFEE, TMHMM, SignalP, PSortB, PDB, Pfam, KEGG, MetaCyc, and Phylogeny). They are expected to understand how these databases work, their limitations, and how they are used to manually annotate genes. Each student is assigned a 20-kb region surrounding a gene of interest to annotate. The entire class also works together to annotate genes encoding a specific metabolic pathway. Annotations are carried out as homework assignments; however, one class period each week is reserved for the students to annotate their assigned genes in the classroom, where we work together as a peer-assisted community to make informed annotation calls [48,49].

Another important goal of this course is for the students to learn to analyze and critically evaluate their annotations in order to develop testable hypotheses that they then address experimentally in the laboratory. Because of the time limitations of a college semester, the students typically will use and assess annotation data from the previous year’s course to develop hypotheses that they then test in wet laboratory experiments. In the first 2 weeks of the laboratory portion of the course, students work in pairs to learn basic microbiological techniques, methods to assess chemotaxis in *P. putida* F1, and they carry out additional literature searches to assist in the design of independent research projects based on annotation data. During the final 2 weeks of the course, students prepare poster presentations that describe the results of their individual annotations, a class-wide poster about the metabolic pathway annotation, and each research pair prepares a poster or oral presentation of their independent research project findings. All posters and talks are presented to the entire department at the Department of Biology Senior Symposium. Each year, some students from BIOL464 continue working on annotation and/or experimental projects for credit or are funded by the NSF grant to work on the chemotaxis project in the laboratory.

This course has evolved over the 4 years that it has been taught mostly in response to inevitable but manageable challenges in its implementation. One of the main challenges from
the instructor’s point of view has been keeping up with the technology, as bioinformatics tools and current databases are continually being upgraded and new databases are being developed. This need drove the implementation of student-based presentations of each database based on the latest publications. In the laboratory, experimental troubleshooting and revising protocols to make them more accessible to larger student numbers has also been a challenge, as some experimental procedures do not translate well from the research laboratory to the instructional laboratory. For example, the development of an easily implemented, interpreted, and documentable assay to assess chemotaxis was required for the more inexperienced students. From the student’s perspective, the greatest challenge that was encountered was striking the balance between a lack of confidence and overconfidence in making annotation calls. This drove a pedagogical change in the classroom such that the annotation of genes of known function (based on published biological activity) was introduced earlier in the semester. As a result, the students were more experienced in the process when they were later required to annotate genes of unknown function.

Results from Student Work

The following section provides an example of the type of data obtained in this project. In the Bioinformatics class, students annotated the putative chemoreceptor gene \texttt{Pput\_3489} and the surrounding 20-kb regions to identify potential operon structure, putative function of nearby genes, gene synteny with other bacterial genomes, and receptor function based on known functions of similar receptors in other organisms (Fig. 1). Based on comparative homology with MCP receptors known to function in amino acid chemotaxis in \textit{Pseudomonas aeruginosa}, gene \texttt{Pput\_3489} was hypothesized to encode a receptor for amino acids, as the product of \texttt{Pput\_3489} was found to be 63% identical to \texttt{PctA} and \texttt{PctB} and 58% identical to \texttt{PctC}. \texttt{PctA}, \texttt{PctB}, and \texttt{PctC} share overlapping specificity for the detection of all 20 amino acids in \textit{P. aeruginosa} PAO1 [27]. The initial studies completed in the course were expanded into undergraduate research projects in both laboratories; one graduate student constructed mutant strains and cloned the \texttt{Pput\_3489} gene [51], and three undergraduates conducted the physiological experiments with mutant strains to demonstrate that \texttt{Pput\_3489} encodes a receptor for amino acids. Previous studies had shown that the presence of the energy taxis receptor \texttt{Aer} can mask mutant chemotaxis phenotypes in swim plate assays [25], and therefore, three mutant strains were constructed. In one strain, \texttt{Pput\_3489} was deleted (\texttt{\Delta Pput\_3489});
TABLE I
Comparison of amino acid receptor specificities in Pseudomonas species

| Amino Acid       | Pput_3489 | PctA<br>
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<tr>
<td>Alanine</td>
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<td>+</td>
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<tr>
<td>Arginine&lt;br&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
<td></td>
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<tr>
<td>Asparagine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cysteine</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Glutamate&lt;br&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>+</td>
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<tr>
<td>Glutamine</td>
<td>+</td>
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<tr>
<td>Glycine</td>
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<tr>
<td>Histidine</td>
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<tr>
<td>Isoleucine</td>
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<tr>
<td>Leucine</td>
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<tr>
<td>Lysine&lt;br&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>+</td>
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<tr>
<td>Methionine</td>
<td>+</td>
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<tr>
<td>Phenylalanine</td>
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<td>+</td>
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<tr>
<td>Proline</td>
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<tr>
<td>Serine</td>
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<tr>
<td>Threonine</td>
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<td>Tryptophan</td>
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<td>Tyrosine</td>
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<td>Valine</td>
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* Data from Pseudomonas aeruginosa PA01 [27].
<sup>b</sup> Not an attractant for P. putida F1.

in the second strain, the gene encoding the energy taxis receptor was deleted (Δaer2); and in the third strain, both genes were deleted (ΔPput_3489/Δaer2) [51]. As a negative control, a strain lacking the essential chemotaxis signal transduction gene cheA was inactivated (cheA::Km). This strain, while still motile, is unable to respond to any external stimuli [26].

P. putida F1 was previously shown to respond to 17 amino acids [51]. Using quantitative swim plate assays, undergraduate students were able to show that the mutant strain that lacks gene Pput_3489 was defective in responding to asparagine and glutamine and that the defect could be complemented with the wild-type Pput_3489 gene expressed in trans (Fig. 2; on previous page). Interestingly, PctA specifically detects asparagine and only PctB detects glutamine in P. aeruginosa [27], indicating that specificity of the MCP encoded by Pput_3489 differs from those of the homologous P. aeruginosa amino acid receptors. Further analyses demonstrated that the Pput_3489 deletion mutant lost the ability to respond to serine, phenylalanine, and tyrosine and had a reduced response to alanine, cysteine, isoleucine, methionine, threonine, and tryptophan (data not shown). When compared with the receptor specificities of PctA, PctB, and PctC (Table I), it is clear that the product of Pput_3489 responds to a unique set of amino acid attractants. These results also indicate that additional chemoreceptors for amino acids are present in P. putida F1; current undergraduates in both the BIOL464 course and the research laboratory are focusing on the identification and characterization of these receptors and on the annotation of amino acid utilization pathways in P. putida F1.

### Student Outcomes

The main goal for the development of this project has been to immerse students in the scientific process in terms of designing, implementing, and analyzing experiments in the context of a genuine scientific question. To assess student outcomes for the BIOL464 course, UST has used data from student ratings of learning on relevant objectives culled by The IDEA Center in which teaching effectiveness is assessed by progress on important and essential course objectives designated by the instructor and overall ratings based on student perception of the instructor and the course (http://www.theideacenter.org). The BIOL464 course and laboratory have been assessed by this method for 3 years. The three main course objectives chosen by the instructor as essential for student learning are as follows: 1) learning fundamental principles, generalizations, or theories pertaining to the field of bioinformatics, 2) learning to apply course material to improve thinking, problem solving, and decisions, and 3) learning to analyze and critically evaluate ideas, arguments, and points of view. These skills have been shown to lead to increased undergraduate and graduate retention in the sciences [52,53]. As shown in Fig. 3, a total of 26 students who completed the course over the 3-year period reported significant learning gains in these three categories. In addition, students self-reported that they made significant gains in skills that were not directly emphasized by the instructor over the course of the semester, which included learning how to find and utilize resources for problem solving. Students also felt as though they had developed essential skills needed by professionals in the field of bioinformatics (Fig. 3).

In addition to learning skills in the classroom, the development of scientific communication skills is important for students in the sciences [54]. Although not all students continue onto careers in research, this experience also contributes by improving the scientific literacy of the general public. More importantly, in addition to experience in communicating research findings at the UST Biology Department Research Symposium and the UC Davis Undergraduate Research Day, a subset of the undergraduate students are provided the opportunity to present at regional and national scientific conferences. To date, a total of four undergraduate UST students and one UC Davis undergraduate have presented their findings at
regional and national meetings, including both poster and oral presentations at the North Central Branch American Society for Microbiology Meeting (2010 and 2011) and poster presentations at the Annual Meeting of the American Society for Microbiology (2010 and 2011) and at the Symposium on Excellence in Nurturing Undergraduate Research Meeting (North Dakota State University Chemistry and Biochemistry Department, 2012). In addition, three UST undergraduates have spent 1–2 months carrying out summer research at UC Davis as part of the project. To date, students directly supported by the project have gone onto both graduate and professional schools, including the Ph.D. programs in Microbiology at the University of Wisconsin and the University of California, Riverside and at the University of Minnesota School of Dentistry.

Conclusions

Here, we have described one example of how utilization of the IMG-ACT annotation infrastructure can be used to involve undergraduates in the process of NSF-funded scientific inquiry. The development of the Bioinformatics lecture and wet laboratory course, and the undergraduate student data mining of the *P. putida* F1 genome and the wet-lab designed and conducted therein, has served as the foundation for further undergraduate and graduate student-independent research projects that are ongoing in our respective research laboratories. The results of student research have taught us that the chemotaxis system in *P. putida* F1 is mediated by a complicated system of receptors with overlapping specificities and that comparative genomics studies with similar pseudomonads can be helpful in identifying the functions of specific receptors. Continued functional genomics studies are expected to reveal additional details about the complex chemotaxis system in this organism.

One of the most important challenges in the development of meaningful undergraduate research experiences is finding ways to balance the time required for teaching and immersing undergraduates in the scientific method with the need to move the science forward in a timely manner. We feel that the example provided here is an excellent combination of undergraduate data mining in the classroom that is paired with individual undergraduate and graduate student research projects that keep the project current and relevant and produces publishable findings. Although the project presented here has been supported by outside funding, this by no means is required for the incorporation of such a course into the curriculum. Where this funding has been most beneficial has been in the financial ability to enable students from a primarily undergraduate institution to conduct research at a research-intensive institution. Although these opportunities clearly cannot be extended to all students that partake in the Bioinformatics course, they do allow for a subset of students genuinely interested in graduate studies to interact with graduate students and postdoctoral associates at UC Davis. The students then bring their experiences back to UST to disseminate their expertise to future undergraduate students working on the project.

Current example course and laboratory syllabi along with additional course materials and examples of student poster presentations funded by this project can be found at the https://sites.google.com/site/pseudomonasputidaf1 website under Instructional Resources and Student Research, respectively. Additional information about incorporating IMG-ACT into the science curriculum is available at the Microbial Genome Annotation Network website (http://mgan-network.org).

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References


