

Teaching Soil Bacterial Diversity from a Phylogenetic Perspective: A Term Project Utilizing the Ribosomal Database Project

Andrew Ogram*

ABSTRACT

Recent advances in the phylogenetic analysis of microorganisms are forcing microbial ecologists to reevaluate ecological and evolutionary relationships among organisms, but these emerging concepts are rarely transferred to students in soil microbiology courses. A term project was designed to familiarize students in an advanced soil microbiology course with the basic concepts of phylogenetic analysis, and to facilitate teaching of soil microbiology from a phylogenetic perspective. Students were requested to select a functional group of soil microorganisms, such as sulfate-reducing bacteria or denitrifying bacteria, and create a tree utilizing the Ribosomal Data Project to show phylogenetic relationships between the various species participating in the function. These trees formed the basis of a final paper and class presentation outlining phylogenetic relationships, the relationship between taxonomy and ecology, and general ecological and biochemical characteristics of members of the groups. Final projects were, for the most part, very high quality. Students' comments were favorable and indicated that the project provided them with an opportunity to utilize Internet resources and obtain a different perspective on the structure of microbial communities.

TEACHING an advanced soil microbiology class directed toward upper division undergraduate and graduate students is challenging, particularly since many students taking these courses have strong backgrounds in traditional soils disciplines such as chemistry and physics, but may be weak in microbiology. I recently developed a course on bacterial diversity in soils as an advanced course for students who had taken the entry level course on soil microbial ecology taught in our department. The objective of the course was to give students an appreciation of the very complex nature of soil microbial communities and to familiarize them with the major taxonomic groups of bacteria that are present in these communities. As part of this course, a final project was designed to familiarize students with modern concepts of taxonomic and evolutionary relationships between groups of organisms.

At first glance, a course on bacterial diversity seems doomed to be rather uninteresting and to require memorization of facts with little opportunity for learning concepts. Memorization of taxonomic groups can be tedious, and bacteria are all basically the same anyway, aren't they? No, bacteria are not all the same; a great diversity exists among soil prokaryotes. One often-cited study indicates that as many as 10 000 species can be present per gram of soil (Torsvik et al., 1994), and their metabolic and genetic diversity speaks

of the roles of these species in soil and of the variability of the soil environment. Evolutionary distances between species and differences in metabolism between different genera rival or exceed similar comparisons among higher eukaryotes.

A perspective is needed that presents bacterial diversity to students in an interesting and meaningful way, such as phylogeny, or the use of evolutionary relationships to order species into taxonomic groupings. By teaching diversity from a phylogenetic perspective, students gain an appreciation of the evolutionary relationships between different groups. They are, therefore, not merely learning the names of bacteria, but are also learning something of how the groups arose. Teaching diversity from a phylogenetic perspective may be thought of as teaching not just the *who* of bacterial diversity, but also some of the *why* and the *how* of diversity. In addition, the language of phylogeny and evolutionary biology is quickly becoming the language of microbial ecology, thanks in large part to the advent of 16S rRNA sequence analysis to define phylogenetic relationships among species (Woese, 1987; Ward et al., 1992; Pace, 1996).

Phylogenetic trees can be relatively straight-forward to build, and a particularly useful tool for building trees is provided by the Internet homepage of the Ribosomal Database Project (RDP) (Maidak et al., 1997). User-friendly programs for creating phylogenetic trees of selected microorganisms are present at this site and are easily incorporated into classroom exercises. This paper describes the use of RDP in generation of phylogenetic trees in the context of a final project for an advanced soil microbiology course. The final class project was designed to give students direct experience with phylogenetic analysis, and therefore, a better understanding of phylogenetic concepts and an appreciation for relationships between soil microorganisms.

Students were asked to develop a natural history of one of a number of functional groups of soil microorganisms. Each student chose a functional group, such as sulfate-reducing bacteria, methanogenic bacteria, or denitrifying bacteria, and investigated the phylogenetic, evolutionary, and ecological relationships between members of the selected group. Important concepts related to rRNA phylogeny and use of RDP were introduced during three classroom periods. A term paper and a brief presentation on this project to the class were required at the end of the semester.

METHODS

The Term Project

Students were asked to select a functional group of soil microorganisms for which they would write a term paper and make a class presentation due at the end of the semester.

Abbreviations: RDP, Ribosomal Database Project; NCBI, National Center for Biotechnology Information.

Soil and Water Science Dep., Univ. of Florida, Gainesville, FL 32611-0290. Florida Agric. Exp. Stn. Journal Series no. R-06188. Received 26 Aug. 1997. *Corresponding author (avo@gnv.ifas.ufl.edu).

Published in *J. Nat. Resour. Life Sci. Educ.* 27:93-96 (1998).
<http://www.agronomy.org/journals/jnrlse/1998/>

Table 1. Creating a tree using RDP.

- Access the RDP homepage (<http://rdpwww.life.uiuc.edu/>). There is no charge or penalty for registration. Note that the URL will change to <http://www.cme.msu.edu/RDP> in December, 1998.
- Under *Analysis Functions*, click on the *Subtree* icon.
- Scroll down to *Selecting the Organisms in the Tree*. You will need to configure a Custom ID list, so click on the hypertext *RDP organism selection page*. This will link to a page entitled *Creating a Custom ID List*.
- Scroll down to the bottom of the page and select the hypertext form in the sentence "Or use the new *form* to type in your own selections." This will link to a new page with *Enter your login name* at the top of your screen.
- Scroll up on this page and choose the hypertext *SSU Prokaryotes in alphabetical order*. SSU refers to the small subunit of the ribosome, or the 16S rRNA database. This is a very large database and may take a few minutes to completely load. Choosing this option will allow you to access the largest number of species in the RDP database. Not all species are present in the database, and it may not be possible using this strategy to include in the tree some species on the student's list.
- Scroll down through the alphabetical list and write down the RDP ShortID names for the species of interest. Selecting the hypertext of each species will display the citation and sequence of the SSU. It is also a good idea to choose a few distantly related genera (outgroups) in your list so that the tree will be more rooted, and the placement of the species in the tree will be more easily related to other bacterial groups.
- Once the Short ID names for all species, including the *outgroups*, have been written down, click *Back* on your browser to return to the *Source Selection* area. Type in a user ID name consisting of 10 characters. The first letter of the genus abbreviation must be capitalized. The exact name is of no importance as it is merely a name that RDP will use to identify your list when creating the tree. Of critical importance, however, is that exactly 10 characters be used—no more and no less than 10.
- Scroll down on this page to the area designated for entering ShortIDs and type in your entries.
- Click on the *Save ID list* button and wait until your user list is displayed.
- Click the *Subtree* icon on the tool bar at the bottom of the screen. This is the icon depicting an axe and a falling tree.
- From the Subtree page, enter your 10-character ID name in the space provided for login name.
- Scroll down on this page and select *Printable* as output formatting. The printable option loses detail on branch lengths, but is easiest to print and modify using a word processor.
- Click the button marked *Submit*, and wait for the tree to be displayed.
- Once the tree is displayed, it may be transferred to a Word or WordPerfect document by highlighting the tree with the cursor, copying the highlighted area, and pasting onto the document of choice.

ter. Topics were to be approved by the instructor approximately 1 mo after the beginning of the semester.

Students were expected to use library and Internet resources to answer the following loosely defined questions regarding their chosen functional group. The directions supplied were intentionally broad so that development of individual projects would stimulate creativity within the subject area:

1. What is the ecological role of the function (e.g., of what importance is denitrification to the nitrogen cycle and to microbial communities)?
2. Analyze phylogenetic relationships between the species and genera involved in the function by placing the species in a phylogenetic tree.
 - a. How many different genera perform the function and do all members of these genera perform the function? (e.g., how many genera are involved in denitrification? Are there *Pseudomonas* species that do not denitrify? Is there more than one genus that denitrifies? If so, what can this tell us of the evolutionary history of the function?)
 - b. Did the function evolve a single time during evolutionary history, or are multiple origins apparent?

Table 2. Student projects.

Denitrifying bacteria
Methanogenic bacteria
Naturally transformable bacteria
Siderophore-producing microorganisms
Sulfate-reducing bacteria
Plant-pathogenic bacteria
Plant-pathogenic fungi
2,4-D degrading bacteria

Developing a Species List

To create a tree using RDP, students should have a list of several species that participate in their chosen function. The number of species chosen is, to a certain extent, dependent on the function. A list of at least 10 species was sufficient to create a tree that would be appropriate for our purposes. Standard text books and review articles may be used to obtain information on the physiology and ecology of the functions, and to develop species lists of bacteria capable of performing the function. *Bergey's Manual of Systematic Bacteriology* (Holt, 1984) and Woese's review of ribosomal RNA phylogenies (Woese, 1987) were also used by many students to obtain more information regarding the phylogeny and physiology of the groups of organisms. A resource that we discovered after the end of the course is located at the National Center for Biotechnology Information (NCBI) web site (<http://www.ncbi.nlm.nih.gov>). The taxonomy browser on the NCBI Taxonomy home page (<http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html>) may be used to quickly identify lineages of genera.

Creating a Tree Using RDP

Much of the help required by students in completing the project was in using the RDP Subtree function to develop the phylogenetic tree. Most of the programs housed by RDP are self-explanatory and are relatively simple to use with little experience, but some familiarity with the programs before use proved valuable. The simplest method we found to create a tree using RDP is presented in Table 1.

It should be noted that we have chosen the *Printable* option for printing the tree. Two other options, *Postwick* and *Newick*, have the advantage that the branch lengths are kept intact showing the approximate distances between the groups. The *Printable* option does not retain meaningful branch lengths. We suggest using the *Printable* option for the students because it is very easy to use, although instructors may wish to use the other options in class for demonstration purposes.

RESULTS AND DISCUSSION

Individual Projects

Students enrolled in the class represented a variety of disciplines and backgrounds. Most students had no experience in microbiology other than the required undergraduate course in soil microbial ecology, although one graduate student had taken several courses in microbiology and had extensive experience in phylogenetic analysis. The students were encouraged to choose topics that were of personal interest to them, and to be creative when considering functional groups (Table 2).

The project was designed to familiarize the students with basic concepts of modern phylogenetic analysis, evolutionary ecology, and the interrelationships between different phylogenetic groups. The primary goal of the project was not that an accurate and detailed evolutionary history be created for the functional group (this would be asking far too much for beginning students!), but rather that the students use the available information to speculate on the interrelationships between species, become familiar with different species, and develop an appreciation for the analysis of microbial diversity.

Use was made of programs supplied by RDP via the Internet (<http://rdpwww.life.uiuc.edu/>) for creating phylogenetic trees of the species involved in the function. Please note that RDP will be moving from the University of Illinois to Michigan State University in December, 1998, at which time the URL will change to <http://www.cme.msu.edu/RDP>. Most background information required for completion of the project could be gathered from easily accessible review articles and text books, but critical thinking was required for analysis of the questions concerning evolutionary history of the species.

An example of a tree showing denitrifying bacteria is presented in Fig. 1. Although this tree presents an incomplete list of species involved in denitrification, the diversity of species is apparent. Most species belong to the α , β , and γ groups of the phylum *Proteobacteria*, but there are also distantly related species in the Gram Positive group. This tree may be placed in the broader context of a tree detailing the various phyla of the domain *Bacteria* for a more general appreciation of the diversity and phylogenetic placement of these species.

Not all microorganisms that have been isolated have been characterized by 16S rRNA sequencing, and not all species that have been sequenced have been processed by RDP. As a result, the RDP database (Version 6.0) is incomplete at the present time, although this limitation is likely to change with subsequent release versions. Some of the species students found for their chosen functions were not present in the current RDP release version and could not be entered into their trees. This did not result in insurmountable problems, however, as many of the species of interest were in the database. In those cases where crucial species were missing from the database, students were usually able to enter a closely related species as a *surrogate* that enabled an approximation of the proper tree to be constructed. Insurmountable problems were encountered, however, when one student was interested in fungi that produce siderophores. Comparatively few fungal 18S rRNA (the rRNA present in the eukaryotic small subunit of ribosomes; comparable to the bacterial 16S rRNA) sequences have been processed by RDP, resulting in a very limited database for fungi. As with the bacterial sequences, these limitations are likely to change as RDP increases the number of sequences processed. Until such time as the fungal database is updated with subsequent releases, this exercise is best conducted with bacteria.

Most students had little trouble making the connection between the phylogenetic relationships of the species involved in the function and the functional groups. The ability to graphically present the distribution of species performing the chosen function allowed the students to mental-

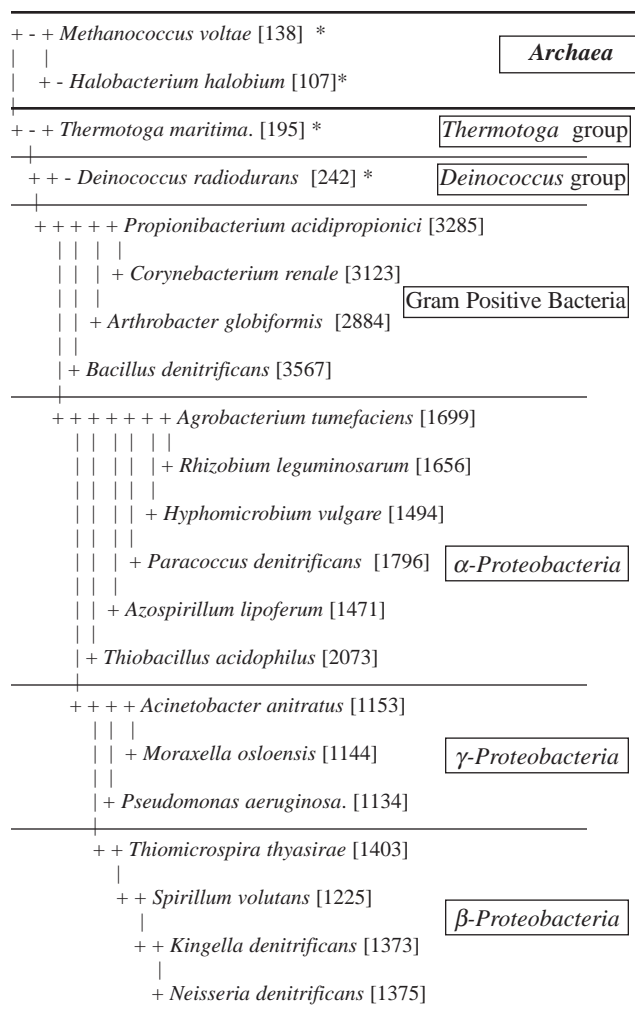


Fig. 1. Phylogenetic tree of denitrifying bacteria. Tree was generated by *Subtree* function of RDP. Asterisks (*) denote nondenitrifying "outgroups" used to aid interpretation of the tree. *Methanococcus voltae* and *Halobacterium halobium* are Archaea. *Thermotoga* and *Deinococcus* are members of the Bacteria, but belong to different phyla than the denitrifying bacteria. Numbers in brackets are RDP tree index numbers. The tree has been annotated by use of a word processor following generation by RDP. Note: This tree is not representative of all genera or species involved in denitrification.

ly map the phylogenetic groups involved in the function, and, we hope, facilitate longer retention than if they were merely given a list of species to memorize.

Students' comments on the project were favorable, and all respondents to the final course evaluation recommended repeating the exercise in future classes. Specific comments include:

I think it was a very good chance to get deeper into a special issue (according to everyone's interests).

Another good thing of the project was to use the internet!!

Its immediate impact was mostly an increased familiarity with Internet resources... I think the project, and the class in general, was a great "stepping stone" for folks with a variety of research interests to begin considering how they might view microbial phylogeny as a tool for better understanding of current microbial activity.

ACKNOWLEDGMENTS

Thanks to bacterial diversity students Hector Castro for helpful discussions concerning methods generation of phylogenetic trees, and John Leader for use of his denitrifying bacteria species list. Thanks especially to Dr. Bonnie Maidak of RDP for many helpful suggestions during the preparation of this manuscript.

REFERENCES

- Holt, J.G. 1984. Bergey's manual of systematic bacteriology. Williams & Wilkins, Baltimore, MD.
- Maidak, B.L., G.J. Olsen, N. Larsen, R. Overbeek, M.J. McCaughey, and C.R. Woese. 1997. The RDP (Ribosomal Database Project). *Nucleic Acids Res.* 25:109–111.
- Pace, N.R. 1996. New perspective on the natural microbial world: Molecular microbial ecology. *ASM News* 62:463–470.
- Torsvik, V., J. Goksoyr, F.L. Daae, R. Sorheim, J. Michalsen, and K. Salte. 1994. Use of DNA analysis to determine the diversity of microbial communities. p. 39–48. *In* K. Ritz et al. (ed.) *Beyond the biomass*. John Wiley & Sons, New York.
- Ward, D., M. Bateson, R. Weller, and A. Ruff-Roberts. 1992. Ribosomal RNA analysis of microorganisms as they occur in nature. *Adv. Microbial Ecol.* 12:219–286.
- Woese, C. 1987. Bacterial evolution. *Microbiol. Rev.* 51:221–271.◆