Catalyzing rapid discovery of gold-precipitating bacterial lineages with university students

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Intriguing and potentially commercially useful microorganisms are found in our surroundings, and new tools allow us to learn about their genetic potential and evolutionary history. Engaging students from different disciplines and courses in the search for microbes requires an exciting project with innovative but straightforward procedures and goals. Here we describe an interdisciplinary program to engage students from different courses in the sampling, identification, and analysis of the evolutionary trajectories of a unique yet common microbe, *Delftia* spp. A campus-wide challenge was created to discover new lineages of this genus, able to precipitate gold, involving introductory level environmental and life science courses, upper-level advanced laboratory modules taken by undergraduate students (juniors and seniors), graduate students, and staff from the campus. The number of participants involved allowed for extensive sampling while undergraduate researchers and students in lab-based courses participated in the sample processing and analyses, helping contextualize and solidify their learning of the molecular biology techniques. The results were shared at each step through publicly accessible websites and workshops. This model allows for rapid discovery of new *Delftia* lineages and is adaptable to different campuses and questions.

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Abstract

Intriguing and potentially commercially useful microorganisms are found in our surroundings, and new tools allow us to learn about their genetic potential and evolutionary history. Engaging students from different disciplines and courses in the search for microbes requires an exciting project with innovative but straightforward procedures and goals. Here we describe an interdisciplinary program to engage students from different courses in the sampling, identification, and analysis of the evolutionary trajectories of a unique yet common microbe, *Delftia* spp. A campus-wide challenge was created to discover new lineages of this genus, able to precipitate gold, involving introductory level environmental and life science courses, upper-level advanced laboratory modules taken by undergraduate students (juniors and seniors), graduate students, and staff from the campus. The number of participants involved allowed for extensive sampling while undergraduate researchers and students in lab-based courses participated in the sample processing and analyses, helping contextualize and solidify their learning of the molecular biology techniques. The results were shared at each step through publicly accessible websites and workshops. This model allows for the rapid discovery of new *Delftia* lineages and is adaptable to different campuses and questions.

Introduction

The potential benefits from the study of the unique abilities of bacteria to everyday human life is ever more obvious (Schlaberg, Simmon, & Fisher, 2012). Bacteria are used industrially in food preparation, drug production, waste treatment, and many other roles. Advances in biotechnology techniques have facilitated the use of known bacterial species and their enzymes, proteins, and pathways (Berini, Casciello, Marcone, & Marinelli, 2017). For example, it is now trivial to identify genes of interest in a bacterial species, clip those genes out of that species, and insert them into another work horse species of bacteria to allow the products of those genes to be produced industrially. Ironically, as our ability to harness the power of bacteria becomes ever more sophisticated, one of the key challenges is still finding the useful bacteria in the first place. In a world with as many as a trillion bacterial species (Locey & Lennon, 2016)(Pike, Viciani, & Kumar, 2018), how does one speed the discovery of bacterial species with a particular use or even simply strains of a particular bacterial taxon with sequences of interest?

One approach is to engage citizen scientists. In as much as the first step in the discovery of novel, useful microbes is often collections from nature, collections made by the public have the potential to speed up this key, and often rate-limiting, first step. What is more, in a rapidly interconnected digital era, the potential for truly global projects that rely on hundreds, thousands, or even hundreds of thousands of individuals is ever greater (Cooper, 2016). Citizen scientists contribute data to many publicly-accessible projects, from birdwatchers helping conservation efforts with the e-Bird project (https://ebird.org/home; Sullivan *et al.*, 2014), game enthusiasts folding proteins for the FoldIt project (https://fold.it/portal/; Cooper *et al.*, 2010), or homeowners exploring the microbial diversity in their houses (http://robdunnlab.com/projects/wild-life-of-our-homes/; Dunn, 2013). Citizen scientists, we argue, can also help discover bacteria with novel, useful traits as well as new strains of bacteria already known to have useful traits.

Arguably, one of the most remarkable bacterial taxa is the *Delftia*, a genus first discovered in the city Delft (Den Dooren de Jong, 1927; Wen, Fega, Hayward, Chakraborty, and

Sly 1999), where bacteria themselves were discovered by Leeuwenhoek (Gest, 2004). *Delftia* have genes capable of precipitating gold (Johnston *et al.*, 2013) with obvious potential uses ("Gold Recycling," 2013; Reith, Lengke, Falconer, Craw, & Southam, 2007; Subhabrata, Natarajan, & Ting, 2017), but to date, the existing diversity of *Delftia* in strain collections is modest. There are only six known species of *Delftia*. Full genome assemblies exist for four of these species within the National Center for Biotechnology Information (NCBI) database (Wen, Fegan, Hayward, Chakraborty, & Sly, 1999). Discovery of novel *Delftia* taxa and their relatives has the potential to better elucidate variations in *Delftia* genetic sequences, especially within the gold precipitation gene cluster and other industrially and human health related sequences. The more information about these gold precipitation genes, for example, the greater potential for using *Delftia* or its genetic potential to recycle our electronics and make mining more sustainable.

Here we leverage a citizen science approach to detect new *Delftia* species on a university campus. We simultaneously test whether students are able to aid the speed of discovery of novel lineages and consider the biology of the lineages we have discovered. The Wolfpack Citizen Science Challenge for spring 2018 (go.ncsu.edu/wpc18) was a university-wide collaborative project to document the presence and genetic diversity of *Delftia* spp. across the North Carolina State University campus and create a *scalable and interdisciplinary* model to continue learning about this and other organisms. In addition to the initial challenge, we also integrated the downstream study of the microbes detected during the Challenge into two university classes.

Materials & Methods

Recruitment of Participants and Sample Collection

The campus-wide challenge was launched with a public event at the James B. Hunt Jr. Library at North Carolina State University on January 30, 2018. The event was publicized broadly to maximize attendance. CCG and NGR shared information about *Delftia acidovorans* found in sinks, drains, and soil, encouraging members of the campus to think critically about the microbial communities around us. Participants were primarily recruited from introductory environmental science courses, but anyone interested was able to obtain a sampling kit and participate. Participants registered as teams of up to five members and were provided kits with instructions and materials to collect samples: three swabs and two 50 mL conical tubes for soil samples along with gloves, plastic spoons for scooping soil, alcohol swabs to sanitize the soil collection spoons, and labels for samples. Approximately forty kits were distributed, and over one hundred and fifty swab and soil samples were received between January 30 and February 14. Samples were delivered in person to either the Biotechnology Program (BIT) teaching laboratories or the NC State University Libraries front desk. Along with physical samples, metadata including location descriptors and latitude-longitude data were submitted online through a customized SciStarter citizen science website (https://scistarter.com/delftia). Students' identifying information was removed from samples and a numerical identity was assigned.

Safety

Participants were provided with detailed instructions on how to sample environments around the campus and use the sampling kit. Participants were instructed to use the swab to sample a safe location and immediately place the swab in the transport container. Soil samples were collected with the provided tube and spoon wearing disposable gloves. For processing of samples, students in molecular biology courses were trained in lab safety procedures and given a

document detailing the potential hazards and safety procedures used in the teaching laboratory. For all extractions and qPCR reactions, students wore provided disposable lab coats, safety glasses, and gloves, and disinfected all surfaces before and after use.

Isolation and Purification of Metagenomic DNA

Metagenomic DNA was extracted from samples using the Invitrogen PureLink Microbiome DNA Purification Kit according to the corresponding protocol for swab and soil samples, respectively. Soil was transferred from collection tubes to bead tubes with alcohol-sterilized metal scoops. Swab tips were cut off into bead tubes with alcohol-sterilized metal scissors. Samples were lysed and homogenized by heat, bead beating, and lysis buffer. After purification, samples were eluted in 50 µl of elution buffer. DNA concentration was determined spectrophotometrically using a ThermoFisher NanoDrop 2000c instrument and normalized to 5 ng/µl. Samples were matched with descriptive location data in an online spreadsheet using information submitted on the SciStarter website (https://scistarter.com/delftia). Isolations were performed by NGR in batches of 12-24 samples.

Detection of Delftia-specific Sequences by Quantitative Real-time PCR

An Eppendorf epMotion 5075 TC liquid handler was used to set up quantitative real-time PCR (qPCR) reactions with New England BioLabs Luna Universal Probe qPCR reagents, primers, and double-quenched probes (IDT DNA). Reactions were set up in duplicate along with an 8-point ten-fold dilution standard curve using purified and quantified amplicon. qPCR reactions were run on a Bio-Rad CFX Connect instrument, and data were exported as spreadsheets with cycle threshold (Ct) values for each reaction. Samples were screened for the quantity of *Delftia* present using double-quenched, *Delftia*-specific primers and probe for a portion of the unique gold biomineralization metabolite production system (hereafter "gold gene"; Johnston *et al.* 2013; GenBank CP000884.1, region 5233319-5234363; see **Appendix 1** for primer and probe sequences, **Seq1, Seq2, Seq3**). Presence and abundance of *Delftia* were then confirmed with a second set of primers and probe for a putative *Delftia*-specific toxin-antitoxin sequence unique to *Delftia* spp. (hereafter "CP sequence"; GenBank CP000884.1, region 759992-760309; see **Appendix 1** for primer and probe sequences, **Seq4, Seq5, Seq6**).

Abundance Estimation of Delftia spp. in Samples

Undergraduate juniors and seniors and first- and second-year graduate students enrolled in an upper-level *High-throughput Discovery* 8-week lab module programmed an epMotion 5075 TC liquid handler with the qPCR script, prepared metagenomic samples for qPCR, and calculated *Delftia* copy numbers using the qPCR Ct data. Students were provided a spreadsheet template with detailed explanations and information on the use of a standard curve for calculation of absolute copy numbers of target sequences. Data were shared with students, and groups of three to four were tasked with determining copy numbers for one 96-well PCR plate containing: twenty-three genomic DNA samples tested in duplicate along with an 8-point standard curve and negative "buffer only" controls. Multiple groups analyzed the same samples to confirm the results, and copy number trends were further supported by analyzing qPCR data for the same samples with a primer set for the single-copy *Delftia*-specific "CP" sequence described above. Data were then analyzed as a class and shared with Danica Lewis (NCSU Libraries) for visualization and dissemination of the results to participants and the public

(go.ncsu.edu/exploredelftia). Samples with the highest *Delftia* copy number using both primer sets were selected for further analysis of the unique "gold" sequence.

Sequencing of "Gold Gene" in Samples Positive for Delftia spp.

For twenty samples with high *Delftia* counts, a portion of the gold gene sequence was amplified using primers **Seq7 and Seq8** identified in **Appendix 1** and the Q5(R) High-Fidelity 2X Master Mix (New England Biolabs) according to the protocol outlined in **Appendix 3**. The amplified portion of the gold gene was selected because it is highly specific to *Delftia* and, based on current sequence database information, varies slightly between known species and strains, allowing for identification from metagenomic samples (see **Appendix 4**). The target *Delftia* sequence is 1045 base pairs in length (**Appendix 5**). Of the twenty tested samples, seventeen produced sufficient PCR product for sequencing and were sent to the NC State University Genomic Sciences Laboratory (GSL) for Sanger DNA sequencing using primers **Seq7 and Seq8**. Amplicons were sequenced from both directions, and sequences were trimmed based on stringent quality settings to match existing sequences in the NCBI database. The sequencing data were shared with the campus community at an event at which participants used the NCBI Basic Local Alignment Search Tool (BLAST) to find regions of local similarity between the discovered sequences and those deposited in the NCBI database. This allowed participants to identify which *Delftia* species and strains best matched the samples that were sequenced (see **Appendices 6-11**).

Data Dissemination

The Google Maps Fusion Tables extension was used to create a heatmap of *Delftia* presence and abundance across campus, and Tableau Public software was used to create an interactive map (http://go.ncsu.edu/ExploreDelftia). Participants were invited to explore the data and evaluate which samples had the highest amount of *Delftia*. Students in the courses involved in sampling and analysis were shown the results and asked to discuss future research questions. Results of the project were shared at a closing event open to campus and the general public, held in Hunt Library on April 11, 2018.

Results

Proportion of Samples Containing Delftia spp. Sequences

Over 150 samples were received from participants. Of these, 135 were labeled correctly and matched with the online SciStarter database containing sampling location descriptions and latitude-longitude coordinates. Through qPCR analysis using primers and probe **Seq1, Seq2, and Seq3**, 125 samples (92.6%) had detectable quantities of the target *Delftia* "gold gene" DNA sequence. Quantities of *Delftia* within samples were confirmed using the CP qPCR primers and probe **Seq4, Seq5, and Seq6**. The twenty samples with highest *Delftia* counts were primarily swabs from sinks and drains (**Table 1**). In contrast, the samples with the least *Delftia* DNA tended to be those from soil samples and outdoor locations. However, it is worth reiterating that nearly all of the samples contained some *Delftia*, a genus of bacteria that one might conclude is rare based on the number of studies in which it is a focus.

We next compared the *Delftia* gold gene sequences in the samples to those of sequenced strains. Collectively, the sequences from our samples were most similar to those of *Delftia tsuruhatensis* strain CM13, *Delftia acidovorans* strains ANG1 and SPH-1, or *Delftia*

acidovorans strain RAY209 (see **Table 2**). Differentiation between *D. acidovorans* strains ANG1 and SPH-1 was not possible as each matched query had the same identity, query coverage, and E value results for both strains. However, for strains of *D. tsuruhatensis* CM13 and *D. acidovorans* RAY209, the sequences matched with highest probability to each, respectively. None of our samples were close matches for the other sequenced *Delftia* species of *D. deserti*, *D. lacustris*, *D. litopenaei*, *D. rhizosphaerae*, or other strains of *D. acidovorans* and *D. tsuruhatensis*.

Fourteen out of the seventeen sequences had less than 97% sequence identity with the *Delftia* strains they most closely matched, suggesting that the majority of the samples on which we focused contained unique and not yet characterized species or strains of *Delftia*. Even relatively conservative approaches regard 97% divergence as sufficient to justify species designation (Schlaberg, Simmon, & Fisher, 2012). Under that criteria, 82% of the samples studied here contained what could be considered new species were the gene we studied to be neutral. The gold gene on which we focused is unlikely to be neutral, such that it cannot be used as a marker of species identify. Nonetheless, the divergence between the strains we documented and known strains is sufficient to allow us to hypothesize that many of these strains are likely to be new species. Regardless of their taxonomic novelty, that these strains are diverse in their gold gene suggests they may have untapped industrial potential.

Discussion

Here, we sought to simultaneously test whether we could engage students, campus wide, in a citizen science style microbial research project and, in doing so, to understand the distribution and diversity of strains of one particular bacterial genus, *Delftia*. In short, we were indeed able to engage students from diverse majors across campus. In doing so, we discovered both that some habitats had many more *Delftia* strains than did others, but also that many of those *Delftia* strains appear to be new to science.

Collectively, the qPCR, Sanger DNA sequencing, and BLAST comparison results showed that strains of *Delftia* are diverse, abundant and frequent (found at many sites) in environments in and around the college campus. Based on available genomic sequences deposited in the NCBI database and partial sequencing of the highly conserved gold gene, the strains students discovered beset matched the reference strains *D. tsuruhatensis* CM13 and *D. acidovorans* ANG1 and SPH-1. However, ten of seventeen samples contained strains that were a 95% or lower match to strains in the NCBI database. In addition, if a unique species or strain of *Delftia* did not contain the specific gold gene sequence investigated, it would be missed in this analysis. Our data indicate that *Delftia* are ubiquitous even in the built environment of the college campus. Importantly, *Delftia* are genetically diverse like many microbes but also understudied. Despite its ubiquity and diversity, the natural history of *Delftia* is poorly understood. Like many bacterial taxa, it is recorded as an opportunistic pathogen that can infect hospitalized or immunocompromised patients (Patel *et al*., 2019)(Bilgin, Sarmis, Tigen, Soyletir, & Mulazimoglu, 2015). However, there is no indication that human bodies are a common habitat for this genus. Instead, it appears to be much more common in soil and water, where its ecological roles (including the importance of the gold gene to its natural history) remain unstudied.

The sequenced *Delftia* gold gene from participant samples matched well to known *Delftia* species, but some samples matched two different existing strains equally well. For example,

samples from 7-1 and 24-1 were equally similar to the strains *D. acidovorans* ANG1 and SPH-1 (**Table 2**). Clearly further work can be done to sequence additional portions or the entire genomes of these samples to identify what known strain is present or discover a new lineage of *Delftia*. More extensive community analyses of the samples using both targeted (16S rRNA gene) and whole genome shotgun sequencing would aid in the identification of which microbes associate with the presence of *Delftia* and the identity of the gold sequences in the environment, respectively. Additionally, high-throughput sequencing approaches such as Hi-C from Phase Genomics ("Hi-C Proximity-Guided Assembly," 2018)(Sieber *et al*., 2018) or Nanopore single-molecule long-read sequencing can be employed to attempt to sequence and assemble the entire *Delftia* genome in metagenomic samples positive for *Delftia* by qPCR. Ultimately, selective media capable of isolating and identifying *Delftia* would allow us to increase our collection of *Delftia* strains for basic functional studies and genome sequencing.

The majority of sequenced gold gene samples shared less than 97% sequence identity with the species and strains in the NCBI database. As a result, the *Delftia* samples we collected potentially contain unique species and strains that have not yet been identified. We were able to identify fourteen sequences with less than 97% identity match. This large number of strains with divergent sequences is almost certainly an indication that there are many unsequenced and yet unknown *Delftia* species and strains, even just in the environments we sampled. These diverse gold gene sequences suggest these new species and strains may have novel industrial potential.

The *Delftia* strains we sequenced best matched the species *D. acidovorans* and *D. tsuruhatensis*, both of which have been found in environments similar to those we studied. *D. acidovorans* was originally discovered in soil and has been found in drains, waterspouts, and showerheads in the built environment (Wen, Fegan, Hayward, Chakraborty, & Sly, 1999). *D. tsuruhatensis* was first discovered in a wastewater treatment plant and has been found in similar locations along with *D. acidovorans* (Hou *et al*., 2015). The *Delftia* species we did not encounter in our study are species that have so far been associated with more restricted habitats. *Delftia deserti* has been found to inhabit desert environments (Li *et al.*, 2015), *D. lacustris* in lake water (Jørgensen, Brant, Nybroe, & Hansen, 2012), *D. litopenaei* in pond water (Chen, Lin, Sheu, & Sheu, 2012), and *D. rhizosphaerae* in the rhizosphere of *Cistus ladanifer*, a plant native to the Mediterranean region (Carro *et al.*, 2017). The apparent ubiquity of the genus *Delftia* hides the reality that individual species appear to show considerable habitat restriction. In the future, it would be interesting to understand which traits and genes of individual *Delftia* species confer the ability to survive in particular habitats.

Our approach kindled campus-wide student interest in microbial diversity and molecular biology techniques through the excitement of discovering new lineages of this unique microbe in places that students frequent on campus. Groups of students from various academic disciplines and courses produced and analyzed samples that contributed to a large public dataset. The findings helped teach the student community about *Delftia*, and also reinforced the importance of the collaborative nature of scientific discovery. The success of this project, in terms of the discovery of new strains of *Delftia* and the documentation of their distribution, helps to validate our general approach. In addition, our approach has the potential to encourage future students to participate. We aim to continue the challenge of finding new *Delftia* lineages and engage others by expanding the sampling opportunity to a multi-section first-year English class that is required for all undergraduate students on our campus. Using a similar approach and incorporating the expertise of faculty in the English department, we will engage students in writing tasks related to the project. Additionally, an upper-level metagenomics course will tie into this endeavor by

processing, sequencing, and analyzing the microbial communities in samples with high numbers of *Delftia* sequences. With relatively minor changes to the course schedules and curricula, one hundred more students per semester can participate, learn, and contribute to the project. We are creating resources that are accessible for other faculty and campuses to implement this project and share findings. For this, students participating in the project are writing the *Delftia* book (go.ncsu.edu/delftiabook), and we have created a group for instructor resources on the QUBES web portal (https://qubeshub.org/community/groups/delftia/projects). Liquid handlers can be cost-prohibitive, but less expensive models such as the Opentrons OT-2 are available, and we are developing scripts for this instrument. Student groups in lab-based courses can always set up qPCRs manually to participate in this project.

Conclusions

As the future plans for integrating this project into courses indicate, enthusiasm for the project was high among our colleagues and grew as the project proceeded. However, if we are to continue the project it is key that it continues to yield new scientific insights. Fortunately, this seems very likely to be the case. For example, although *Delftia* abundance was very patchy on campus, we have yet to explain what factors account for such patchiness. Additional samples will help us to have sufficient coverage across sample types to allow spatial models of *Delftia* diversity and abundance. In addition, it is evident from our samples that many *Delftia* strains remain to be discovered. Conversely, there is a lack of genomic diversity represented in the NCBI database. By leveraging the enthusiasm of university students and staff, interconnecting courses and researchers, and using our model pipeline, new lineages of *Delftia* can be rapidly identified and studied (*e.g.,* groups of students cloning novel gold gene cluster into a host such as *Escherichia coli* or yeast for functional characterization). This will yield a better understanding of the ecological and environmental significance of these organisms and simultaneously help to connect students and faculty across campus in a common scientific project. Finally, it is of note that *Delftia* species, while little known, are of potentially great applied importance. They often contain genes associated with resistance to mouthwash (which have potential consequence for oral ecology). In addition, they contain genes that allow many strains to precipitate gold. Given the many waste streams in which gold is present but hard to concentrate, this ability has the potential to be very useful moving forward.

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Table 1(on next page)

Top twenty samples with *Delftia* DNA identified via qPCR targeting of unique “gold” gene.

Table 1: Top twenty samples with *Delftia* DNA identified via qPCR targeting of unique "gold" gene.

DNA Sample Number	*Delftia* Gold Gene Count	Latitude	Longitude	Sample Type	Location	Description
15-1	113,191	35.78593062	-78.66805315	Swab	Poe Hall	Water fountain
7-1	17,294	35.78472956	-78.67292404	Swab	Owen Residence Hall	*None provided*
24-1	14,167	35.78822065	-78.67522672	Swab	University Towers	parking deck drains
1-3	12,041	35.78654	-78.671737	Swab	Williams Hall	bathroom sink
17-3	9,493	35.78068018	-78.67308866	Swab	Wood Residence Hall	Sink drain
23-2	8,780	35.78468	-78.666723	Swab	SAS building	The girls bathroom sink on the first floor of SAS building, middle sink
33-2	5,789	35.78744498	-78.67013454	Swab	DH Hill Library	3rd floor Women's bathroom sink
12-2	5,095	35.785385	-78.673091	Swab	Metcalf bathroom	bathroom sink
26-1	5,095	35.74477072	-78.68757963	Swab	Campus Crossing	Apartment complex
9-1	4,612	35.78795303	-78.67699295	Swab	Valentine Commons	Kitchen sink
44-4	4,356	35.78670028	-78.67463044	Soil	fence on Dan Allen Dr.	chilly (56 F), drier soil, live organisms present
25-2	2,961	35.7861221	-78.66352558	Swab	NCSU Bell Tower, main campus	Wild Card sample-seat located on NCSU bell tower
45-4	2,317	35.78753054	-78.67083426	Soil	Atrium	Trash bins next to the vending machines
24-2	1,971	35.78822065	-78.67522672	Swab	University Towers	drain
15-2	1,873	35.785982	-78.677831	Swab	Lee Hall	Suite 807 Sink

18-1	1,733	35.78481659	-78.67285967	Swab	Owen residence hall	inside in dorm room
25-1	1,535	35.77153404	-78.67522001	Swab	Engineering Building I, Centennial Campus	Drain in the middle of the floor of the bathroom
29-2	1,452	35.78751407	-78.66981704	Swab	DH Hill	Floor 1
7-2	1,381	35.78412031	-78.67101431	Swab	Talley Student Union	Bathroom Sink Drain
30-2	1,113	35.78824567	-78.67403984	Swab	Nelson Hall	Water fountain

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Table 2(on next page)

NCBI BLAST results for sequenced environmental “gold gene”.

Table 2: NCBI BLAST results for sequenced environmental "gold gene".

Sample	Species and Strain	Identity	Query Coverage	E Value
1-3	*Delftia tsuruhatensis* strain CM13	98%	100%	0.0
9-1		96%	100%	0.0
12-2		95%	99%	0.0
15-1		97%	100%	0.0
15-2		95%	100%	0.0
17-3		95%	100%	0.0
25-1		95%	100%	0.0
25-2		96%	100%	0.0
26-1		96%	100%	0.0
30-2		95%	99%	0.0
33-2		97%	100%	0.0
7-1	*Delftia acidovorans* strain ANG1 *or* strain SPH-1	91%	100%	0.0
18-1		94%	99%	0.0
23-2		95%	100%	0.0
24-1		95%	100%	0.0
7-2	*Delftia acidovorans* strain RAY209	82%	99%	3e-154
30-3		96%	99%	0.0