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Improving the classification of key marine microorganisms for biogeography and ecophysiology studies

This MASTS fellowship supported the visit of Dr. David Berry, assistant professor at the University of Vienna, to Heriot-Watt University to work with the group of Dr. Tony Gutierrez in July and August 2014. The visit facilitated the development of a scientific research project related to the identification of hydrocarbon-degrading bacteria in environmental surveys using sequencing-based techniques. Sequencing-based microbial surveys generate large datasets and can reveal the distribution of microorganisms in pristine and contaminated environments. The performance of these methods, however, remains largely under-evaluated for key environmental microbes such as hydrocarbon-degrading bacteria.

The purpose of our work was to update the phylogeny and taxonomic classification of obligate hydrocarbon-degrading bacterial taxa and to evaluate their detection by state-of-the-art sequence analysis tools. The project was initiated in order to give the scientific field a benchmarked evaluation of the performance of sequence analysis tools and to provide practical recommendations for future studies. As hydrocarbon degraders are key to oil spill bioremediation in marine systems this project is highly relevant to MASTS.

Networking with Scottish Researchers
The visit was highly successful in facilitating interactions with Scottish researchers. During his stay, Dr. Berry visited several Scottish institutes and gave seminars at Heriot Watt University, University of Glasgow, and the Scottish Universities Environmental Research Centre. This

strengthened an ongoing collaboration with Drs. Thomas Preston and Douglas Morrison (Scottish Universities Environmental Research Centre) and also helped to initiate a collaboration with Prof. Harry Flint and Dr. Alan Walker (University of Aberdeen).

In addition, Dr. Berry taught students in the group of Dr. Gutierrez sequence analysis skills and advised them one-on-one on the analysis of data from their PhD research projects. Dr. Berry also engaged in many informal research discussions with members of the the faculty of Heriot Watt during his visit and discussed ideas for joint grant applications.

<u>Research Objectives</u>
The goal of the main research project was to improve detection of obligate hydrocarbon degrading bacteria in sequencing surveys. As part of this, the following steps were necessary:
1. Updating the phylogeny of hydrocarbon degrading taxa
2. Evaluating the suitability of available primer sets for targeting these taxa
3. Evaluating the ability of bioinformatics tools to properly classify these taxa

<u>Initial Results</u>
Obligate oil degrading bacteria are represented by a restricted number of groups, the Gammaproteobacterial genera *Alcanivorax*, *Marinobacter*, *Oleispira*, *Oleiphilus*, *Thalassolituus*, and *Cycloclasticus* as well as the Alphaproteobacterial genus *Thalassospira*. Using comprehensive database of the bacterial 16S rRNA gene, a phylogenetic marker gene, we extracted sequences related to these taxa and their sister taxa for phylogenetic analysis. We generated phylogenetic trees to determine which sequences were monophyletic and could be classified to these taxa with high confidence (Fig. 1).
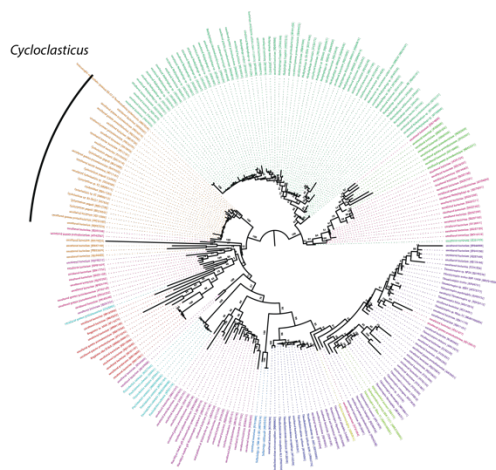

Figure 1. Representative phylogenetic tree of the genus *Cycloclasticus* as well as sister genera.

We then used these validated sequences to compare the coverage of over 100 published primer sets for their coverage of these taxa. This analysis revealed several promising candidate primer sets that had very high (>98% coverage) for these taxa. Furthermore, using the primer set with the highest coverage (S-D-Bact-0343-a-S-15 and S-D-Bact-0908-a-A-18) we tested how well resulting amplicons could be correctly classified using current bioinformatics tools. We found that the amplicons were correctly classified for all target taxa (Table 1), indicating that this primer set is a very promising candidate for future studies targeting hydrocarbon-degrading bacteria.

Table 1: Classification results for validated sequences using the region amplified by primers S-D-Bact-0343-a-S-15 and S-D-Bact-0908-a-A-18 (HV regions 3-5). Amplicons were classified with RDP Classifier 16S rRNA database version 15.

Genus	No. Tested Seqs.	% Correctly classified	% Classified to genus level with 80% confidence
Alcanivorax	732	100	98
Cycloclasticus	61	100	100
Neptunomonas	38	100	100
Oleiphilus	1	100	100
Oleispira	35	100	100
Thalassolituus	19	100	100

Overall Logistics

The work was carried out in the group of Dr. Tony Gutierrez, an expert in hydrocarbon-degrading bacteria. The visit took place during the summer of 2014, when Dr. Berry was able to take a 6 week leave of absence from the University of Vienna.

Communication of Results

We are currently preparing a manuscript from this work and we aim to publish this work in an international peer-reviewed journal in the area of environmental microbiology. The results will also be presented at scientific conferences such as the MASTS Annual Science Meeting (October 2016) and the Society for Applied Microbiology Summer Conference (July 2016).

Significance of Work in Relation to State of the Field, and Potential Benefits

This project provided a much-needed benchmarking of primer sets for sequencing surveys targeting hydrocarbon degraders. The results of this work provides valuable insights for environmental microbiologists that will assist them in the design of future studies.

Acknowledgements and Outlook

I (David Berry) would like to thank MASTS for providing the financial support that allowed me to visit the lab of Dr. Gutierrez. This was a very productive 6 weeks and led to a lot of exchange

within his group, at Heriot Watt University, and with other Scottish research institutes. As an outlook, we hope that the publication resulting from this work will help to form a basis for a joint grant application to acquire European funding.