BISMiS-2016
Abstract Book

Third Meeting of

Bergey's International Society for Microbial Systematics

on Microbial Systematics and Metagenomics

September 12-15, 2016 | Pune, INDIA

MCC
सूक्ष्मजीव संवर्धन संकलन
MICROBIAL CULTURE COLLECTION
National Centre for Cell Science, Pune

BISMiS 2016

ASSOCIATION OF MICROBIOLOGISTS OF INDIA 1938
PUNE UNIT

Abstracts

- Opening Address
- Keynotes

Opening Address

TAXONOMY OF PROKARYOTES - NEW CHALLENGES IN A GLOBAL WORLD

Peter Kämpfer*
Justus-Liebig-University Giessen, HESSEN, Germany
Email: peter.kaempfer@umwelt.uni-giessen.de

Systematics can be considered as a comprehensive science, because in science it is an essential aspect in comparing any two or more elements, whether they are genes or genomes, proteins or proteomes, biochemical pathways or metabolomes (just to list a few examples), or whole organisms. The development of high throughput sequencing techniques has led to an enormous amount of data (genomic and other "omic" data) and has also revealed an extensive diversity behind these data.

These data are more and more used also in systematics and there is a strong trend to classify and name the taxonomic units in prokaryotic systematics preferably on the basis of sequence data. Unfortunately, the knowledge of the meaning behind the sequence data does not keep up with the tremendous increase of generated sequences.

The extent of the accessory genome in any given cell, and perhaps the infinite extent of the pan-genome (as an aggregate of all the accessory genomes) is fascinating but it is an open question if and how these data should be used in systematics. Traditionally the polyphasic approach in bacterial systematics considers methods including both phenotype and genotype.

And it is the phenotype that is (also) playing an essential role in driving the evolution. The criteria used for systematics may change, when we have a full insight into the complexity of the genomes (and the "phenome") of microorganisms.

However, the maintenance of stable, workable and predictable taxonomic and nomenclatural standards is even more important in these times of "high-throughput" techniques.

Keynote 1

USE OF GENOME SEQUENCE DATA IN BACTERIAL TAXONOMY: PERSPECTIVES FROM LARGE SCALE ANALYSIS

Jongsik Chun*
School of Biological Sciences and ChunLab, Inc., Seoul National University, Seoul 08826, Republic of KOREA
Email: jchun@snu.ac.kr

Next generation sequencing (NGS) and accompanying bioinformatics has lowered the technical and economical barriers that have prevented microbiologists from using genome sequence data in various research areas, including bacterial taxonomy. The genome is the ultimate source of information that can be used in all taxonomic levels, from defining new phyla to resolving the transmission of a bacterial clone between patients. Even though it is clear that genomics will play a key role in classification and identification of Bacteria and Archaea, the detailed methodology is not well established.

As of July 2016, almost 70,000 genomes, as assemblies, are available in public database for Bacteria and Archaea of which ~98% are bacteria. In addition, a large quantity of genome data is also being accumulated as a form of raw data generated by conventional and new NGS systems. As in the case of 16S rRNA gene database, data and metadata of genome database for taxonomic use should be carefully curated and timely updated. It is also crucial to develop adequate and affordable (in terms of computing cost) bioinformatics tools, either as standalone software or web-services.

In this talk, I will review the bioinformatics strategy for genome-based classification and identification, and introduce new integrated database for 16S rRNA gene and genome sequences (EzBioCloud). In addition, new web-based tool to detect possible contamination of genome data, named ContEst16S (http://tool.ezbiocloud.net/contest16s/) will be introduced.

The presentation file can be requested to jchun@chunlab.com.

Keynote 2

CULTIVATION-INDEPENDENT GENOMICS APPROACHES AND THEIR RELEVANCE TO MICROBIAL TAXONOMY

B.P. Hedlund*
School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV 89154, USA
Email: brian.hedlund@unlv.edu

A vast diversity of Bacteria and Archaea exists in nature that has evaded axenic culture. For example, meta-analysis of high-throughput 16S rRNA gene surveys of terrestrial geothermal systems worldwide (n = 372) suggest very high relative abundances of yet-uncultivated phyla ($\bar{x}$ = 16.8%), classes ($\bar{x}$ = 34%), orders ($\bar{x}$ = 42.1%), and families ($\bar{x}$ = 46.9%) [1]. Single-cell genomics, metagenomics, and a variety of molecular microbial ecology approaches provide ever-improving insight into the biology of these organisms; however, due to the *International Code of Nomenclature of Prokaryotes*, yet-uncultivated microorganisms are not accommodated in formal taxonomy regardless of the quantity or quality of data. Meanwhile, efforts to calibrate the existing taxonomy with phylogenetic anchors and genomic data are increasingly robust. The current climate provides an exciting opportunity to leverage rapidly expanding single-cell genomics and metagenomics datasets to improve the taxonomy of Bacteria and Archaea. However, this opportunity must be weighted carefully in light of the strengths and limitations of these approaches. We favor use of the *Candidatus* taxonomy to include taxa that are described genomically, particularly when genomic work is coupled with advanced molecular ecology approaches to probe metabolic functions *in situ* [2]. This system preserves the rigor and value of traditional microbial systematics while enabling growth of a provisional taxonomic structure to facilitate communication about "dark" lineages on the tree of life.

References

[1] Hedlund, B.P., Murugapiran, S.K., Alba, T.W., Levy, A., Dodsworth, J.A., Goertz, G.B., Ivanova, N., Woyke, T. (2015) Uncultivated thermophiles: current status and spotlight on 'Aigarchaeota'. *Current Opinion in Microbiology* 25:136-45.

[2] Hedlund, B.P., Dodsworth, J.A., Staley, J.T. (2015) The changing landscape of microbial biodiversity exploration and its implications for systematics. *Systematic and Applied Microbiology* 38:231-236.

Keynote 3

COMMUNITY WIDE INSIGHTS INTO STRESSED NICHES USING METAGENOMIC APPROACH

Rup Lal*
Molecular Biology Laboratory, Department of Zoology, University of Delhi, Delhi 110007, India
Email: ruplal@gmail.com

Here, we have utilized the potential of stressed niches in terms of analyzing diversity at species and strain level resolution to further understand the population dynamics via metagenomics. We have looked into two significantly stressed sites i.e. Hexachlorocyclohexane (HCH) dumpsite (450 mg HCH per g soil) and Himalayan hot springs (surface temperature > 95°C). At HCH contaminated sites, the combination of the genomes of two genetic subspecies (*Sphingobium japonicum* UT26 and *Sphingobium indicum* B90A) capable of degrading HCH, with metagenomic data enabled the reconstruction and validation of the last-common ancestor (LCA) genotype. Along the same line at the Hot spring sites, we were able to reconstruct two novel genomes of potential predator (*Bdellovibrio bacteriovorus*) and prey (*Enterobacter cloacae*). These data were used to construct a theoretical model describing potential predator avoidance strategies, whereby the *E. cloacae* strains can move between anaerobic and aerobic niches by quorum sensing population size, which is modulated by a 'kill the winner' viral mechanism and predation by the obligate aerobe, *B. bacteriovorus*.

Keynote 4

THE VALUE OF CULTURES TO MODERN MICROBIOLOGY

B. Austin*
Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK
Email: baustin5851@gmail.com

Since the late nineteenth century, the acquisition of pure bacterial cultures has been central to all facets of microbiology. Microbial pathology is no exception insofar as the goal of disease diagnostics and research is the acquisition of pure cultures of the pathogen. The basic premise is that pathological material may be inoculated onto a solid [usually gelled with agar] or into a liquid medium with incubation for a pre-determined interval when individual cells of the pathogen will be cloned into dense culture growth, which will then be subjected to further study. However, the range of media used by bacteriologists is restricted, and often centres on nutrient agar/broth, tryptone soya agar/broth and/or brain heart infusion agar/broth. Moreover, the incubation regimes may have little relevance to the growth conditions of the diseased animal. However, the desired outcome is the presence of dense virtually pure growth, which is taken as indicative of recovery of the pathogen. Unfortunately at best, a snap shot of the disease is obtained, and it may not be possible to decide if only one organism instigated the infection, and then contributed to the development of overt disease signs. It is unlikely that culturing on a single occasion would identify microbial population succession within a disease cycle. Also, conventional techniques are unlikely to recognise when two or more discrete organisms working synergistically to produce a single pathology. This situation has been observed with ulcerative conditions in cyprinids when *Aeromonas salmonicida* and *A. hydrophila*/*A. sobria* may be involved together, with the former instigating infection, and the latter leading to the developing of large ulcers. Certainly, it is realized that not all cells will multiply sufficiently to produce visible colonies. Some cells produce micro-colonies that are invisible to the naked eye. Moreover, the proportion of culturable cells that produce visible growth will vary according to the species and the state of the cells – are they actively growing or comparatively inactive? The latter have a poorer rate of recovery in terms of culturability. The next premise is that an individual colony is derived from multiplication of a single cell. Yet, it is realized that cells in close proximity to each other may multiply and come together to produce a single colony. Then, the resultant growth will most certainly be derived from more than one initial cell. This has greater relevance if the two initial cells are from two different species. Although it is generally assumed that streaking and re-streaking on fresh media will purify any culture, there is evidence for microbial consortia interacting to form what appear to be single pure cultures. Thus, seemingly pure cultures of purple-pigmented aquatic bacteria were recognized to contain cells of *A. salmonicida*. As so-called pure cultures underpin most of microbiology, it is relevant to understand that the culture does not necessarily contain clones of identical bacteria, but that there is a variation in the genetic potential of the component cells, i.e. the cells are not homogeneous. Certainly, many bacteria change rapidly upon culturing in the laboratory. Cells may become bigger and less active in the laboratory, i.e. genetic potential is lost. It is difficult to be sure if the changes reflect a loss of DNA or whether standard culturing methods select faster growing cells that are effectively not representative of the environment from which they were derived.

Keynote 5

MEGACULTUROMICS OF MICROBIAL BIODIVERSITY FROM DIVERSE ECOLOGICAL NICHES IN INDIA

Y.S. Shouche*
Microbial Culture Collection, National Centre for Cell Science, SP Pune University, Pune 411007, Maharashtra, India
Email: yogesh@nccs.res.in

Microbial bioprospecting is the process of discovery and commercialization of new products based on biological resources. A growing need for new bioactive compounds in the pharmaceutical, agricultural and food industries stresses the importance of prospecting for novel bio-active compounds. In our study, approximately 2, 40,000 bacterial isolates were screened for production of various bio-active compounds from diverse ecological niches throughout India. In order to capture the maximum microbial diversity, 30 specific culture media were used for isolation. In order to assess total bacterial diversity captured, we randomly identified 15,000 bacterial isolates by using 16S rRNA gene sequencing and MALDI-TOF based methods. Apart from ubiquitous organism like *Pseudomonas* and *Bacillus* etc. many bacterial species were found to be specific for culture medium as well as to the ecological niche. Many diverse bacterial species with potential to produce various bio-active compounds could be acquired with this strategy. Our results suggest that selection of specific media and diverse ecological niches are important factors for grasping maximum bacterial diversity. Based on our results from this megaculturomics approach, here we suggest combination of media and ecological niches to acquire maximum possible bacterial diversity using culture based approach. Phylogenetic analysis based on 16S rRNA gene sequences revealed that highly similar organisms with variation in culturing conditions and habitat may show completely different physiological and biochemical properties which in turn may be related with the production of different bioactive compounds (metabolite).

Keynote 6

NOVEL INSIGHTS INTO MICROBIAL SYSTEMATICS BASED ON MOLECULAR ECOLOGY AND COMPARATIVE GENOMICS APPROACHES

Jörg Overmann*
Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstraße 7 B, 38124 Braunschweig, Germany
Email: Joerg.Overmann@dsmz.de

The extent of microbial diversity, and the mechanisms maintaining it, can only be fully appreciated if the evolving units within the prokaryotic radiation can be delineated. In this respect, advanced high-throughput sequencing technology in combination with novel cultivation methods have improved our perception of bacterial diversity and systematics. For example, a high-resolution, culture-independent analysis of habitat adaptation within soil *Acidobacteria* across multidimensional environmental gradients revealed an unexpected multitude of ecological niches and previously unknown adaptations for the supposedly functionally redundant bacteria. During evolution, differential habitat adaptation within clades of phylogenetically closely related lineages was frequently disrupted by switches in habitat preferences. These data provide the basis for the isolation and characterization of the first representatives of subgroup 6 *Acidobacteria* by high throughput cultivation methods. Systematic comparisons of closely related bacterial genomes provide further insights into the genomic basis of differentiation. Thus, an analysis of marine *Phaeobacter* sp. revealed a large core genome with a stable phylogeny and very little recombination, ongoing genome expansion, and a specific niche adaptation of different subclades that correspond to different species despite a 16S rRNA gene similarity of > 99.5%. Similarly, comparative analysis of freshwater *Sphingomonadaceae* revealed extraordinary high levels of nucleotide diversity between similar phylotypes, that are sustained by high population mutation rates but low recombination rates. The incipient sexual isolation of subpopulations is caused by natural selection rather than genetic drift or demographic effects. Discrete seasonal abundance patterns were detected based on high-throughput sequencing of internal transcribed spacer sequences in natural samples, indicating a selective advantage for individual subpopulations in the natural environment. In combination, these approaches will help to refine the prokaryotic species concept, are the preconditions for an improved bioeconomical exploitation of microbial diversity, and affect the perception of the uniqueness of microbial ressources in the framework of international treaties such as the Nagoya Protocol.

Keynote 7

MODEST PROPOSALS FOR UNIFICATION OF THE NOMENCLATURE OF CULTURED AND UNCULTURED PROKARYOTES

William B. Whitman*
Department of Microbiology, University of Georgia, Athens, GA 30602-2605, USA
Email: whitman@uga.edu

A consistent and unambiguous nomenclature is essential for scientific communication. The International Code of Nomenclature of Prokaryotes (or the Code) initially developed during the last century to address this need in Bacteriology. Originally patterned after the Botanical Code, a number of unique features developed after 1980. Of special importance are the requirements for registration of all new names with the International Committee for Systematics of Prokaryotes and for the type material to be a living, axenic culture. When these criteria are met, the name is given priority depending upon the date of registration. In this fashion, the Code insures that each taxon has only one name and the name corresponds to real biological entity. However, modifications of the Code are necessary to incorporate recent breakthroughs in molecular ecology and genomics in the nomenclature. First, when gene sequences unambiguously identify a species, they should be allowed to serve as the type material. This change of the Code will allow naming many fastidious and symbiotic prokaryotes that cannot be currently deposited in culture collections. Moreover, the names will remain correct even if the type strains are lost from culture collections. Second, when gene sequences unambiguously identify a genus, they should be allowed to serve as the type material in the absence of named species. This change of the Code will allow the unification of the nomenclature of the cultured and uncultured prokaryotes into a single robust system. The candidatus system of nomenclature would then no longer be necessary and could be abandoned. In this fashion, the Code will remain independent of any particular taxonomic philosophy, the type material will provide a clear identification of the taxon, and nomenclature will remain current with the developments in modern biology.

Keynote 8

THE CURRENT STATUS OF CYANOBACTERIAL NOMENCLATURE UNDER THE "PROKARYOTIC" AND THE "BOTANICAL" CODE

A. Oren*
The Institute of Life Sciences, The Hebrew University of Jerusalem, Edmond J. Safra Campus, Jerusalem 91904, Israel
Email: aharon.oren@mail.huji.ac.il

At the meetings of the ICSP in Sydney in 1999, General Consideration 5 of the International Code of Nomenclature of Prokaryotes (ICNP) was, more or less by mistake, modified to include the cyanobacteria. This modification of the Prokaryotic Code has proven highly problematic, and the nomenclature of the group was traditionally covered by the International Code of Nomenclature for algae, fungi, and plants (ICN), and the two codes are not always compatible [1]. While the ICN recognizes names validly published under the ICNP, Article 45(1) of the ICN was not (yet) reciprocated by the ICNP. Not all cyanobacterial experts are aware of the ways the codes work, and erroneous statements can be found in the taxonomical literature based on the assumption that the two codes regulate taxonomy, phylogeny and classification [2]. Different solutions have been proposed to solve the current problems [1,3]. In 2012 a Special Committee on the harmonization of the nomenclature of Cyanobacteria between the ICN and the ICNP was appointed, but this committee was dissolved in 2015 as its activity has been minimal. Two opposing proposals to regulate cyanobacterial nomenclature were submitted, one calling for deletion of the group from the ICNP [4], the second to consistently apply the rules of the ICNP [5]. These proposals will be on the agenda of the upcoming meeting of the ICSP.

References

[1] Oren, A. and Tindall, B.J. (2005) Nomenclature of the Cyanophyta/Cyanobacteria/ Cyanoprokaryotes under the International Code of Nomenclature of Prokaryotes. *Algological Studies* 117: 39-52.

[2] Oren, A. (2015) Comments on "Taxonomy of cyanobacteria: a contribution to consensus approach", by K. A. Palinska and W. Surosz (Hydrobiologia 740: 1-11, 2014). *Hydrobiologia* 758: 1-2.

[3] Oren, A. (2015) Comments on: "A polyphasic approach leading to the revision of the genus *Planktothrix* (Cyanobacteria) and its type species, *P. agardhii*, and proposal for integrating the emended valid botanical taxa, as well as three new species, *Planktothrix paucivesiculata* sp. nov.^{ICNP}, *Planktothrix tepida* sp. nov.^{ICNP}, and *Planktothrix serta* sp. nov.^{ICNP}, as genus and species names with nomenclature standing under the ICNP, by V. Gaget, … and N. Tandeau de Marsac. *Systematic and Applied Microbiology* 38: 159-160.

[4] Oren, A. and Garrity, G.M. (2014) Proposal to change General Consideration 5 and Principle 2 of the International Code of Nomenclature of Prokaryotes. *International Journal of Systematic and Evolutionary Microbiology* 64: 309-310.

[5] Pinevich, A.V. (2015) Proposal to consistently apply the International Code of Nomenclature of Prokaryotes (ICNP) to names of the oxygenic photosynthetic bacteria (cyanobacteria), including

those validly published under the International Code of Botanical Nomenclature (ICBN)/International Code of Nomenclature for algae, fungi and plants (ICN), and proposal to change Principle 2 of the ICNP. *International Journal of Systematic and Evolutionary Microbiology* 65: 1070-1074.

Session Abstracts

- Genomic/Metagenomic Description of Novel Taxa
- Cultures and Culturing of as-yet-uncultivated Microbes
- The Role of Cultures in the Twenty First Century
- Modern Approaches to Identification/ Diagnosis
- Minimum Standards for the Description of New Taxa
- Cyanobacterial Taxonomy

Session 1 – Genomic/Metagenomic Description of Novel Taxa

COMPARATIVE GENOMIC ANALYSIS AMONG MEMBERS OF *STAPPIACEAE* FAM. NOV. AND PROPOSAL OF TWO NOVEL *LABRENZIA* SP.

D.R. Arahal*, L. Rodrigo-Torres, A. La Mura and M.J. Pujalte
Spanish Type Culture Collection (CECT) & Dept. of Microbiology and Ecology; University of Valencia, Paterna 46980, Valencia, Spain
Email: arahal@uv.es

The genera *Labrenzia*, *Nesiotobacter*, *Pannonibacter*, *Polymorphum*, *Pseudovibrio*, *Roseibium* and *Stappia*, are formaly included into the family *Rhodobacteraceae* (*Rhodobacteraceae sensu lato*) [1] in spite of being distant from the clade formed by the true members of *Rhodobacteraceae* (*Rhodobacteraceae sensu stricto*). Indeed they could be considered a separate family, *Stappiaceae* fam. nov., containing aerobic or facultative anaerobic chemoorganotrophs, with important roles on bioactive compounds production, carbon monoxide oxidation, heavy metal decontamination and crude degradation. Their main habitats are aquatic environments, such as marine water and sediments. They have also been found in fresh water, thermal springs, saline soils, and associated to algae and marine invertebrates [1].

In order to provide a better description with the formal proposal of this novel taxon we performed a comparative genomic analysis. Thus, we accomplished the *de novo* sequencing of a few strains available at the Spanish Type Culture Collection to complement those already available at public databases (NCBI/ENA/DDBJ) from these genera. Most of the approaches employed have been already tested in recent studies in our research unit [2-4]. Additionally, this work permitted us to propose two novel *Labrenzia* species, named *L. algae* and *L. pulchra*.

References

[1] Pujalte, M.J., Lucena, T., Ruvira, M.A., Arahal, D.R., Macián, M.C. (2014) The family *Rhodobacteraceae*. In: Rosenberg, E., DeLong, E.F., Stackebrandt, E., Lory, S., Thompson, F. (ed), The Prokaryotes—*Alphaproteobacteria* and *Betaproteobacteria*, 4th ed, vol 8, pp. 439-512. Springer Verlag, Berlin, Germany.

[2] Rodrigo-Torres L, Pujalte MJ, Arahal DR. (2016) Draft genomes of *Nautella italica* strains CECT 7645^T and CECT 7321: Two roseobacters with potential pathogenic and biotechnological traits. *Mar Genomics* 26:73-80.

[3] Rodrigo-Torres L, Pujalte MJ, Arahal DR. (2016) Draft genome sequence of *Thalassobius mediterraneus* CECT 5383^T, a poly-beta-hydroxybutyrate producer. *Genom Data* 7:237-9.

[4] Arahal DR, Pujalte MJ, Rodrigo-Torres L (2016) Draft genomic sequence of *Nereida ignava* CECT 5292^T, a marine bacterium of the family *Rhodobacteraceae*. *Stand Genomic Sci*. 11:21.

BISMiS-2016 International Student Travel Award Recipient

GENOME BASED PHYLOGENETIC ANALYSIS TO INVESTIGATE RELATIONSHIPS BETWEEN THE GENERA *ERWINIA, PANTOEA* AND *TATUMELLA*

M. Palmer*, E. T. Steenkamp, M. P. A. Coetzee, T. A. Coutinho, S. N. Venter
[1.] *Department of Microbiology and Plant Pathology and the Genome Research Institute, University of Pretoria, Pretoria 0002, South Africa*
[2.] *DST-NRF Centre of Excellence in Tree Health Biotechnology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa*
Email: marike.duplessis@fabi.up.ac.za

The investigation of the evolutionary relationships between related species and genera with a variety of lifestyles has gained popularity in recent years. This is because genome-based approaches may provide insight into how specific traits evolve in bacteria. Obtaining a robust evolutionary history is, however, essential before biological inferences can be made. In this study we examined the evolutionary relationship between the closely related genera *Erwinia*, *Pantoea* and *Tatumella* as previous gene based phylogenetic studies have proposed different evolutionary hypotheses for these genera. To accomplish this, shared genes were identified from 43 genomes, representing 34 different species belonging to these three genera, as well as 9 outgroup taxa. Phylogenomic analyses were performed and results were compared to the conventional multi-locus sequence analysis (MLSA) approach. Factors generally associated with phylogenetic incongruence were also investigated. We found that the nucleotide datasets employed in MLSA and the core genome dataset contained significant levels of substitution saturation and differential codon usage, both of which likely gave rise to the observed lineage specific rate heterogeneity. The combined impact of these effects was evident in the incongruent topologies that were obtained using different tree reconstruction methods. The effects of these factors were much less pronounced in the amino acid dataset of the core genome and could be accounted for by employing maximum likelihood tree building approaches with appropriate substitution models for individual gene partitions. This generated a robust and well-supported evolutionary hypothesis for the three genera which confidently resolved the relationships among *Erwinia*, *Pantoea* and *Tatumella*. Future use of comparative genomics combined with phylogenomics will improve our understanding of the drivers of these genera and will undoubtedly inform sound taxonomic decisions.

GENOMIC INSIGHTS INTO THE SPECIES *Micromonospora saelicesensis* AND ITS INTERACTION WITH LEGUMES

Martha E. Trujillo*
Depto de Microbiología, Universidad de Salamanca, 37007 Salamanca, Spain.
E-mail: mett@usal.es

The genus *Micromonospora* is a Gram-stain positive, filamentous actinobacterium that is widespread in diverse habitats. In the last years *Micromonosporae* have been reported as major components of nitrogen fixing root nodules of both leguminous and actinorhizal plants [1,2]. A recent study (see poster by Benito et al., 2016) has also confirmed that presence of *Micromonospora saelicesensis* in other parts of the plant, including leaves, stems and roots. While the species diversity in this niche is very high, the species *Micromonospora saelicesensis* appears to be the most abundant species found in many of the plants sampled hitherto.

In the present study the genomes of several strains identified as *Micromonospora saelicesensis* were sequenced in order to gain further insight into their relationship with plants and potential use as a plant growth promoters. In addition, the possiblity to analyze several strains of the same species, has provided a good basis for looking into the intraspecific variability.

References

[1] Trujillo, M. E., Alonso-Vega, P., Rodríguez, R., Carro, L., Cerda, E., Alonso, P., and Martínez-Molina, E. (2010). The genus *Micromonospora* is widespread in legume root nodules: the example of *Lupinus angustifolius*. *ISME J.* 4, 1265-1281.
[2] Carro, L., Pujic, P., Trujillo, M.E.,and Normand, P. (2013a). *Micromonospora* is a normal inhabitant of actinorhizal nodules. J Biosci 38: 685–693.

LESSONS LEARNT FROM THE USE OF GENOMES IN THE DESCRIPTION OF NEW SPECIES OF *AEROMONAS* AND *ARCOBACTER*

Maria José Figueras*
Unit of Microbiology, Department of Basic Health Sciences, Faculty of Medicine and Health Sciences, IISPV, University Rovira i Virgili, Sant Llorenç 21, 43201 Reus, Spain.
Email: mariajose.figueras@urv.cat

Aeromonas and *Arcobacter* are two genera of bacteria found in water environments and that embrace species considered to be emergent human pathogens. In our laboratory we have discovered 38.7% of the described *Aeromonas* species (12/31) and 43.5% (10/23) of the ones of *Arcobacter*. In fact we have introduced, for the first time, genome information derived from the use of the Average Nucleotide Identity (ANI) and the *in silico* DNA-DNA hybridization (*is*DDH) in the description of new *Aeromona* species. This was extremely useful because it helped to determine more objectively if new candidate species recognized on the basis of a Multilocus Phylogenetic Analysis (MLPA) of five housekeeping genes represented indeed new species. Now we are doing the same for describing several new *Arcobacter* species. In addition both the ANI and the *is*DDH showed to be extremely friendly and useful tools. However, we were confronted with some important problems we had to solve. First, despite many genomes of *Aeromonas* were available at the GenBank, when we started, most of them did not correspond to the ones of the type strains of the different species. Furthermore we discovered that 36% of the deposited *Aeromonas hydrophila* genomes did not correspond to this species. Therefore we propose strategies to validate the true identity of the genomes [1, 2]. Despite of that, the mislabelled genomes keep on being mislabelled at the GenBank. The second surprise was the variability of results obtained using the different platforms available for the calculation of the ANI, which in some cases could be misleading [1, 2]. Despite of this, the cut-off value for species differentiation using ANI was determined for the genus *Aeromonas* at 96% [1, 2]. The genomes of the type strains of the *Arcobacter* spp. are also missing and this is a problem we are trying to solve now sequencing these genomes. Here, we were confronted with chimeric genomes of which only one was evident due to the bigger genome size, while another required assembly with different tools to show up size differences. The sequences of the housekeeping genes that lead us to suspect that the strains corresponded to new species were combined with the same genes extracted from the newly obtained genomes in phylogenetic trees for validation of the DNA identity. This is now the quality control strategy we are using in our laboratory to recognise chimeras. Therefore standardized control criteria are needed to guarantee a homogeneous quality for the genomes deposited at the different databases and used for describing new species.

References

[1] Beaz-Hidalgo, R., Hossain, M.J., Liles, M.R. and Figueras, M.J. (2015) Strategies to avoid wrongly labelled genomes using as example the detected wrong taxonomic affiliation for *Aeromonas* genomes in the GenBank database. *PLoS One* 10(1):e0115813.

[2] Figueras, M.J., Beaz-Hidalgo, R., Hossain, M.J. and Liles, M.R. (2014) Taxonomic affiliation of new genomes should be verified using Average Nucleotide Identity and Multilocus Phylogenetic Analysis. *Genome Announcements* 2(6). pii: e00927-14.

INTEGRATION OF AVERAGE AMINO ACID IDENTITY (AAI) AND PERCENTAGE OF ORTHOLOGOUS GENES (BBH) IN A SINGLE PHYLOGENOMIC METRIC, THE RECIPROCAL ORTHOLOGY SCORE AVERAGE (ROSA).

A.N.Gale, J.E.Krebs, E.M.Peluso , J.D.Newman*
Lycoming College, Williamsport, PA 17701, USA
Email: newman@lycoming.edu

With the decreasing cost of NextGen sequencing and the subsequent increase in the availability of microbial genome sequences, it has been suggested that the prokaryotic species definition should change from physical measurements of DNA-DNA hybridization (DDH) to computationally determined genome-wide metrics. The Reciprocal Orthology Score Average (ROSA) metric described here is calculated using Average Amino Acid Identity (AAI) and percent bidirectional best-hit (%BBH) genes at its core. We have developed a JavaScript-based tool (http://lycofs01.lycoming.edu/~newman/ROSA.html) that calculates AAI, %BBH, and ROSA using the output from the SEED Viewer Sequence-Based Comparison Tool on the Rapid Annotation with Subsystems Technology (RAST) service (http://rast.nmpdr.org). The ROSA metric has a range from below 3 when comparing genomes from different domains to above 99 when comparing closely related strains. Organisms at every taxonomic level from subspecies to domain are clustered more accurately using ROSA thresholds than with any existing published metric because it takes into consideration both similarity of orthologs as well as percentage of the genome composed of orthologs.

A DATABASE PROTOTYPE FOR GENOME-BASED DESCRIPTIONS OF ANY KIND OF MICROBIAL TAXA

B. A. Vinatzer*, L. S. Heath, L. Tian, G. Hughes, H. A. El Marakeby
Virginia Tech, Blacksburg, VA 24061, USA
Email: vinatzer@vt.edu

Genome sequencing and whole genome phylogenetic reconstruction has revealed a substantial genetic diversity within many bacterial species and allows to classify bacteria to a depth unimaginable in the past. Current taxonomy was never intended to classify bacteria to this depth and strain typing methods, such as multilocus sequence typing, do not provide a framework for consistent naming of infraspecific bacterial groups. To address these challenges and to take full advantage of genome sequencing for the description of bacterial diversity we have proposed to assign codes to individual genome-sequenced organisms whereby codes reflect whole genome similarity among organisms [1]. We call these codes Life Identification Numbers (LINs). Besides enabling the naming of individual strains, LINs can also be used to describe groups of related bacteria. Groups could represent a named genus or species but could also be as genetically restricted as a single population associated with a disease outbreak. A prototype LIN database and website is currently being developed that will allow submission of bacterial genome sequences and metadata, automated assignment of LINs, and the possibility for users to add descriptions and names to any group of related genomes. We expect this database to greatly simplify description of bacterial taxa, be it a named species or any other group of related bacteria.

References

[1] Marakeby, H., Badr, E., Torkey, H., Song, Y., Leman, S., Monteil, C.L., Heath, L.S., Vinatzer, B.A. (2014) A system to automatically classify and name any individual genome-sequenced organism independently of current biological classification and nomenclature. *PLOS ONE* 9(2):e89142 doi: 10.1371/journal.pone.0089142

K-SHUFF: A NOVEL ALGORITHM FOR CHARACTERIZING STRUCTURAL AND COMPOSITIONAL DIVERSITY IN GENE LIBRARIES

Kamlesh Jangid[1,2]*, Ming-Hung Kao[3], Aishwarya Lahamge[2,4], Mark A. Williams[5], Stephen L. Rathbun[6], William B. Whitman[1]

[1] Department of Microbiology, University of Georgia, Athens, Georgia, USA; [2] Microbial Culture Collection, National Centre for Cell Science, SP Pune University, Pune, Maharashtra, India; [3] School of Mathematical & Statistical Sciences, Arizona State University, Tempe, Arizona, USA; [4] Institute of Biotechnology and Bioinformatics, SP Pune University, Pune, Maharashtra, India; [5] College of Agriculture and Life Sciences, Virginia Polytechnic and State University, Blacksburg, Virginia, USA; [6] Department of Epidemiology and Biostatistics, University of Georgia, Athens, Georgia, USA.

E-mail: jangidk@nccs.res.in

K-shuff is a new algorithm for comparing the similarity of gene sequence libraries, providing measures of the structural and compositional diversity as well as the significance of the differences between these measures. Inspired by Ripley's *K*-function for spatial point pattern analysis, the Intra *K*-function or IKF measures the structural diversity, including both the richness and overall similarity of the sequences, within a library. The Cross *K*-function or CKF measures the compositional diversity between gene libraries, reflecting both the number of OTUs shared as well as the overall similarity in OTUs. A Monte Carlo testing procedure then enables statistical evaluation of both the structural and compositional diversity between gene libraries. For 16S rRNA gene libraries from complex bacterial communities such as those found in seawater, salt marsh sediments, and soils, *K*-shuff yields reproducible estimates of structural and compositional diversity with libraries greater than 50 sequences. Similarly, for pyrosequencing libraries generated from a glacial retreat chronosequence [1] and Illumina® libraries generated from US homes [2], *K*-shuff required at least 300 and 100 sequences per sample, respectively. Power analyses demonstrated that *K*-shuff is sensitive to small differences in Sanger or Illumina® libraries. This extra sensitivity of *K*-shuff enabled examination of compositional differences at much deeper taxonomic levels, such as within abundant OTUs. This is especially useful when comparing communities that are compositionally very similar but functionally different. *K*-shuff will therefore prove beneficial for conventional microbiome analysis as well as specific hypothesis testing.

References

[1] Jangid, K., Whitman, W.B., Condron, L.M., Turner, B.L., and Williams, M.A. (2013). Soil bacterial community succession during long-term ecosystem development. *Mol Ecol* 22: 3415-3424.

[2] Dunn, R.R., Fierer, N., Henley, J.B., Leff, J.W., and Menninger, H.L. (2013). Home life: factors structuring the bacterial diversity found within and between homes. *PLoS One* 8: e64133.

Session 2 – Cultures and Culturing of as-yet-uncultivated Microbes

ISOLATION OF NOVEL METHANOTROPHS FROM INDIAN RICE RHIZOSPHERE INCLUDING A PUTATIVE NEW GENUS WITHIN TYPE Ia METHANOTROPHS

Monali Rahalkar* and Pranitha Pandit
Bioenergy Group, MACS Agharkar Research Institute, Pune- 411004
Email: monalirahalkar@aripune.org

Cultivation and isolation of methanotrophs is important but challenging. Rice rhizospheres and roots are the hotspots of methanotroph activities dominated by 'Type Ia' methanotrophs [1]. Molecular approaches have suggested the presence of several genera of Type Ia methanotrophs; however, to date the cultivated species from rice fields belong to only *Methylomonas* genus. Combination of strategies were used to enrich and isolate the most dominant methanotrophs from a rhizospheric sample of a flooded rice field in India. Strategies included: use of a dilute mineral medium, serial dilution till extinction enrichment in microtitre plates, low oxygen, agarose as solidifying agent and longer incubation times. Three putative novel methanotroph species were isolated from the enrichment. One of the unique isolates, strain Sn10-6, obtained from the last positive grown dilution enrichment (10^{-6}) was found to be a member of a putative novel genus within Type Ia methanotrophs. Sn10-6 is a large, pale-pink pigmented, cucumber shaped and motile, which would be one of the largest methanotroph being reported so far (3.5-5 µm to 1.2–1.5 µm). Draft genome of Sn10-6 showed many important characters that might be helping its survival in the rhizosphere e.g. nitrogen fixation, Type IV pili, motility and chemotaxis genes [2]. Metagenomics of the rhizosphere indicated that the methane monooxygenase genes showed close relationship with that of Sn10-6 *pmoA*, revealing its significance in the environment [3]. Presently, formal characterization of Sn10-6 is ongoing and would be proposed as a novel genus and species as *Methylocucumis oryzae* gen. nov. sp. nov.

References

[1] Shrestha M, Abraham WR, Shrestha PM, Noll M & Conrad R (2008) Activity and composition of methanotrophic bacterial communities in planted rice soil studied by flux measurements, analyses of pmoA gene and stable isotope probing of phospholipid fatty acids. *Environ Microbiol* **10**: 400-412.

[2] Rahalkar MC, Pandit PS, Dhakephalkar PK, Pore S, Arora P & Kapse N (2016) Genome characteristics of a novel Type I methanotroph 'Sn10-6' isolated from a flooded Indian rice field. *Microb Ecol* **71**: 519-523.

[3] Pandit PS, Rahalkar M, Dhakephalkar P, Ranade DR, Pore S, Arora P & Kapse N (2016) Deciphering community structure of methanotrophs dwelling in rice rhizospheres of an Indian rice field using cultivation and cultivation independent approaches. *Microb Ecol* **71**: 634-644.

PRESERVATION OF UNCULTURED IN OMICS ERA

Om Prakash*
Microbial Culture Collection, National Centre for Cell Science, SP Pune University, Pune 411007 Maharashtra, India
Email: omprakash@nccs.res.in

Current data on cultivation indicated that despite all efforts only 1-10% microbial diversity has been cultured and preserved for academic as well as commercial exploration. Remaining 90 - 99%; with immense agricultural, medicinal, and biotechnological potential are yet to be brought into culture, and unavailable for future use and exploitation. Rapid development of next generation sequencing (NGS) platforms have made sequencing easy, affordable and less time consuming which attracted microbiologist towards culture independent omics approaches like metagenomics, metaproteomics, metatranscriptomics and metabolomics. Efforts for cultivation of uncultured microbes have become less attractive due to time consuming and difficult nature of microbial cultivation and preservation. Although, culture independent omics data has revealed the presence of several novel group of organisms and solved several myths about microbial diversity but it has its own limitations. This approach only tells about the structural aspect of the microbial community, but; unable to study the functionality and biotechnological importance of the microbes. Thus, cultivation is necessary for study of functionality of microbes and to understand their ecological relevance and commercial exploitation. In addition, modern theories in ecology has proven that like plant and animals microbes are also facing the problem of extinction and endanger due to ecological perturbations and climatic variations. It is therefore essential to preserve them for future generation. Considering the importance of culture several innovative methods have been developed to cultivate the novel organisms but it is impossible to cultivate them in short period of time and there is a need to preserve the intact microbiome of ecological relevance for future culturomics, metagenomics, genomics and single cell biology. In my presentation; I will discuss the innovative method and protocols used for the cultivation of not yet cultured bacteria and also provide a brief overview of advances in intact microbiome preservation for future culturomic, metagenomics and single cell biology study.

Session 3 – The Role of Cultures in the Twenty First Century

EXTENDING CHARACTERIZED DIVERSITY TO SHAPE THE TAXONOMIC STRUCTURE OF THE ORDER *PLANCTOMYCETALES*

S. Dedysh*
Winogradsky Institute of Microbiology, Research Center of Biotechnology RAS, Leninski Ave. 33/2, Moscow 119071, Russia
Email: dedysh@mail.ru

The order *Planctomycetales* and the family *Planctomycetaceae* were created two decades ago in order to accommodate two genera of morphologically unique, gram-negative, budding bacteria, the *Planctomyces* and *Pirella* (now known as *Pirellula*) [1]. Since that time, the described diversity within the *Planctomycetaceae* has significantly expanded. At present, this family includes 16 genera with validly published names, which belong to four major phylogenetic lineages defined by the genera *Isosphaera*, *Gemmata*, *Pirellula,* and *Gimesia*. The 16S rRNA gene sequence identity between members of different lineages is in the range of 77-85%. Only the phylogenetic lineage defined by the genus *Isosphaera* was recently given the status of a family, i.e. *Isosphaeraceae* fam. nov. [2]. Members of the *Planctomycetales* possess large genomes (5-10 Mb) and more than half of their predicted proteins are of unknown function [3, 4]. Further progress in understanding the biology and metabolic potential of these bacteria largely depends on our ability to cultivate and comprehensively characterize these unique bacteria since many of them are slow-growing organisms that are difficult to manipulate in the laboratory. The chemotaxonomic analysis of planctomycetes is a highly challenging task because these bacteria contain many unique lipids, which cannot be identified using routine techniques. Therefore, a combination of genome analyses and laboratory experiments with cultures is needed for shaping the taxonomic structure of the order *Planctomycetales*.

References

[1] Schlesner, H. and Stackebrandt, E. (1986). Assignment of the genera *Planctomyces* and *Pirella* to a new family *Planctomycetaceae* fam. nov., and description of the order *Planctomycetales* ord. nov. *Systematic & Applied Microbiology* 8: 174-176.
[2] Kulichevskaya, I.S., Ivanova, A.A., Suzina, N.E., Rijpstra, I.W.C., Sinninghe Damsté, J.S. and Dedysh, S.N. (2016) *Paludisphaera borealis* gen. nov., sp. nov., a hydrolytic planctomycete from northern wetlands and the proposal of *Isosphaeraceae* fam. nov. *IJSEM* 66: 837-844.
[3] Jogler, C., Waldmann, J., Huang, X., Jogler, M., Glıckner, F.O., Mascher, T., Koltera, R. (2012) Identification of proteins likely to be involved in morphogenesis, cell division, and signal transduction in *Planctomycetes* by comparative genomics. *Journal of Bacteriology* 194: 6419-6430.
[4] Guo, M., Zhou, Q., Zhou, Y., Yang, L., Liu, T, Yang, J. and 10 other authors. (2014) Genomic evolution of 11 type strains within family *Planctomycetaceae*. PLoS ONE 9(1): e86752.

DISCOVERING NOVEL BACTERIA WITH AN EYE TO BIOTECHNOLOGICAL APPLICATIONS

Syed G Dastager*
NCIM Resource Center, CSIR-National Chemical Laboratory, Pashan Road, Pune-4110008, Maharashtra, India.
Email: sg.dastager@ncl.res.in; syedmicro@gmail.com

Microbial diversity provides not only the basics for human life but also for the life support systems of the biosphere. However, the conservation of microbial diversity has not been given the due importance. It is appropriate now, to account the present status of microbial diversity in improving the human welfare and sustainable development. Microorganisms play a role in conservation and restoration biology of higher organisms, and microbial communities are excellent models for understanding biological interactions and evolutionary history. New technologies are being developed that are based on diverse organisms, from diagnostics to biosensors and to biocatalysts. Much more needs to be done, however, on how to understand better the microorganisms, inventory their diversity, maintain reference cultures of them, and find ways to exploit them for biotechnological benefit. The observation that, microorganisms are valuable natural resources for industry is not new. The importance and myriad applications of microorganisms are well known and human beings have been making microorganisms work for them for a very long time, even before knowing the basic facts about microorganisms. The microorganisms are providing a vast array of products for the welfare of the human kind and the success of microbial biotechnology relies on the diversity of microorganisms and their systematics. The untapped diversity of microorganisms is a resource for new genes and organisms of value to biotechnology. An attempt was made to understand the diversity of actinomycetes/actinobacteria and their biotechnological importance. The alkaline soils, sediment samples obtained from different niches of the region have given the potential candidate isolates of actinomycetes/actinobacteria and many Bacillus species. The biosystematics, based on the advanced chemical and molecular approaches, discloses novelty of the isolates. The bio-potentials applications for various enzymes and plant growth promoting attributes with novel candidates were well established.

References

[1] Dastager. S. G., Wen-Jun Li, Dayanand.A, Sulochana.M.B. Shu-Kun Tang., Xin-Peng Tian, and Xiao-Yang Zhi. (2007). *Streptomyces gulbargensis* sp.nov., isolated from Karnataka soils. India. Antoine van Leeuwenhoek. Vol 91: 99-104.

[2] Dastager. G. Syed Dayanand. Agasar, Wen-Jun Li, Chang-Jin Kim Jae-Chan Lee , Dong-Jin Park, Xin-Peng Tian and Raziuddin.Q.S (2008) Proteolytic activity from an alkali thermotolerant *Streptomyces gulbargensis* sp.nov, Current Microbiology, 57:638-642.

[3] Dastager. G. Syed, Jae Chan Lee, Wen-Jun Li, Chang-Jin Kim and Dayanand Agasar (2009). Production, characterization and application of Keratinase from *Streptomyces gulbargensis*. Bioresource Technology-(100):1868-1871.

[4] Dastager. G. Syed, Dayanand Agasar and Ashok Pandey (2009). Production and partial purification of α-amylase from a novel isolate *Streptomyces gulbargensis*. J. Industrial Microbiology and Biotechnology. 36: 189-194.

[5] Syed. G. Dastager, Raziuddin.Q.S, Deepa C.K, Wen-Jun Li and Ashok Pandey (2010) *Pontibacter niistensis* sp. nov., isolated from forest soil in India. Int J Syst Evol Microbiol.60:2867-2870.

[6] Syed. G. Dastager, C.K. Deepa and Ashok Pandey (2011) Plant growth promoting potential of *Pontibacter niistensis* in cowpea (*Vigna unguiculata* (L.) Walp.). Applied Soil Ecology. 49; 250– 255.

[7] Syed. G. Dastager, Zheng- Liu Qiang, Samir Damare, Shu-Kun Tang and Wen-Jun Li (2012). *Agromyces indicus* sp. nov., isolated from mangroves sediment in Chorao Island, Goa, India. Antonie van Leeuwenhoek.102:345-352.

[8] Syed. G. Dastager and Samir Damare (2013). Marine Actinobacteria showing Phosphate solubilizing efficiency in Chorao Island, Goa, India. Current Microbiology 66:421-427.

[9] Syed G Dastager, Rahul Mawlankar, Shan-Kun Tang, Krishnamurthi Srinivasan, V. Venkata Ramana, Neeta Joseph, and Yogesh S. Shouche (2014). *Rhodococcus enclensis* sp nov., new member of *Rhodococcus* genus. IJSEM. 64:2693-2697.

[10] Priya. S. Neurgaonkar, Mahesh. S. Dharne, Syed. G. Dastager (2016) Draft Genome Sequence of *Arthrobacter enclensis* NCIM 5488^{T} for Secondary Metabolism. May/June 4(3): 3 e00497-16

A STUDY OF PROKARYOTIC DIVERSITY FROM MARINE HABITATS OF THE CENTRAL WEST COAST OF INDIA USING CONVENTIONAL AND METAGENOMICS APPROACH.

S. Krishnamurthy*
Microbial Type Culture Collection, CSIR-IMTech, Chandigarh, India
Email: kmurthi@imtech.res.in

An attempt has been done to study the prokaryotic diversity from marine habitats of the Central West coast of India by using the culture dependent and independent approaches. The area of Western Ghats, being one of biodiversity hotspots, harbours some of the islands and beaches with minimal anthropogenic perturbations, and offers an excellent source of prokaryotic diversity. A total of eight sampling points targeting four mangrove areas of the state of Goa and intertidal coastal macroalgae of the West coast (four beaches in the state of Goa and Maharashtra) were selected for the analyses. A total of about 720 isolates from the mangrove sediments and as epiphytic bacteria of the macroalgae have been purified and preserved (based on morphology) by plating the samples on eleven different media to take care of diverse phylotypes i.e., oligotrophic bacteria. For preliminary characterizations, about 244 isolates were randomly selected and screened through phenotypic tests, partial sequencing of 16S rRNA, whole cell MALDI-TOF profiling and fatty acid based semi-automated identification systems. On a whole, culture-dependent analyses revealed that majority of the isolates were placed in the phyla, *Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes*. Out of the characterized isolates about fifteen strains seem to be novel at the level of genus and species and these are in the process of detailed polyphasic taxonomic characterization. In order to have a holistic approach on the prokaryotic diversity of these habitats, metagenomics approach was applied using next generation Illumina sequencing targeting the V3-V4 region of the 16S rRNA gene. Preliminary findings from the data reveal a common and complex pattern of diversity of bacterial populations (separately for sediment and algal samples) with predominance of the phylum *Proteobacteria* followed by *Bacteroidetes, Firmicutes, Actinobacteria, Chloroflexi, Nitrospirae, Fusobacter, Verrucomicrobia, Cyanobacteria, Tenericute*s while some sequences were found to be unclassified at various taxonomic levels. Interestingly we have been successful in isolating many isolates whose sequences have been retrieved in metagenomics. Currently we are in the process of further analyzing the data including selection of interesting strains (which might play a role in the ecological process of the habitat inclusive of the abundant taxa retrieved in the metagenomics) and will pursue with whole genome sequencing of the isolates. We are hopeful that this project will give us useful and important insights into the microbial functions of these critical habitats whose knowledge can be applied for environmental and societal benefits.

DISTRIBUTION, DIVERSITY AND POTENTIAL ACTIVITY OF BACTERIA IN COASTAL AREAS OF THE MEDITERRANEAN SEA AT SPACE AND TIME

K. Richa* and R. Casotti
Stazione Zoologica Anton Dohrn, Villa Comunale, Naples-80121, Italy
Email: kumariricha.szn@gmail.com

Despite the increasing recognition of crucial role of marine microbes in the flux of matter and energy, there is still a lack of knowledge about their phylogenetic and physiological diversity, and the environmental drivers that underlie their distribution and ecology. Coastal areas, characterized by high heterogeneity and productivity, are ideal system for understanding microbial response to the dynamic nature of marine environment. Here we investigated the spatio-temporal distribution and dynamics of bacterial communities, along the coastal areas of the mid-western Mediterranean Sea, with a special focus on the Gulf of Naples (GON), employing flow cytometry and high-throughput sequencing (Illumina) of V4-V5 hypervariable region of the16S rRNA gene. We also assessed the relative potential activity of individual bacterial taxa using the ratio of 16S rRNA to 16S rRNA genes. Results demonstrated that the abundances of autotrophic (*Synechococcus* and *Prochlorococccus*) and heterotrophic bacteria, as estimated by flow cytometry, were strongly regulated by a combination of different environmental factors and complex hydrological features leading to a heterogeneous distribution and dynamics across space and time. Heterotrophic bacteria numerically dominated the overall prokaryotic population while *Synechococcus* were the major Cyanobacteria. High resolution investigation of the taxonomic groups of bacterioplankton communities also showed a high beta-diversity, with the rare phylotypes contributing to a major proportion of the shifts in community composition at short spatial, seasonal and hourly scales. Dominating groups across both space and time (season and hours) were *Alphaproteobacteria* (mostly SAR11 and *Rhodobacteraceae*), *Bacteroidetes* (mostly *Cryomorphaceae* and *Flavobacteriaceae*) and *Gammaproteobacteria* (mostly *Alteromonadaceae* and SAR86). Our study revealed biogeography in community structure of bacteria in the GON which were related to their response to the local and large scale environmental flux and also suggested that the individual taxonomic groups might be adapted to distinct niches. The relationship between potential activity and abundance was positive for the overall bacterial community but when individual taxa were assessed, some incidences of uncoupling were evident, with many of the rare taxa exhibiting higher potential activity than the abundant ones. This study highlighted the relevance of 'rare' phylotypes in the GON and bacterial signatures that can be treated as markers of specific ecological feature and can also be used to compare with those present in other coastal areas with similar features.

Session 4 – Modern Approaches to Identification/Diagnosis

IN-SILICO CHEMOTAXONOMY: A TOOL FOR 21ST CENTURY MICROBIAL SYSTEMATICS.

P.A. Lawson[1]*, K. Sankaranarayanan[1], Nisha B. Patel[1], Hans-Jürgen Busse[2]
[1] *University of Oklahoma, Norman, USA;* [2] *University, Mycology and Hygiene, Vienna, Austria.*
Email: paul.lawson@ou.edu

Currently, the focus of a fierce debate between microbiologists is the very concepts and methods used in prokaryotic systematics and how this should be approached in the future [1]. Opinions vary from the viewpoint of a polyphasic approach in tandem with phylogenetic analyses, to the provision of utilizing only the genome with a few minimal phenotypic traits being described [2, 3]. However, a unifying theme emerges whereby a strong taxonomy must encompass sufficient biological markers to make the assignment of taxa to a particular group a robust process. Information from genomic analysis derived via affordable next generation sequencing must be embraced, but not at the expense of good taxonomic practices. Chemotaxonomic methods are typically labor intensive and impacted by a lack of cross-laboratory expertise and standardization in data generation and reporting and thus have eluded a high throughput approach. The ease of generating whole genome sequences now makes it possible to utilize metabolic pathway information and genome annotation as an alternative tool to examine these important diagnostic biomarkers. Bacterial strains distributed across five phyla, with published genomes will be used as a reference to develop "*in silico"* prediction models. The phyla targeted in this study comprise microbes with clinical, environmental, and broader biotechnological relevance. Genomic data will be compared with published data for chemotaxonomic features; where necessary data and characterization of unknown markers will be generated in-house. Once prediction models are built, they will be evaluated on a separate set of organisms spanning a similar phylogenetic range.

The development of these models and tools (searchable, curated web-based databases) have great potential to revolutionize chemotaxonomy making it accessible to the broader scientific community rather than the relatively small number of specialist laboratory that currently undertake this analysis.

References

[1] Sutcliffe IC, Trujillo ME, Goodfellow M. (2012). The BISMiS 2011 special issue on prokaryotic systematics, a vital discipline entering a period of transition. *Antonie van Leeuwenhoek* 10:1-2.
[2] Oren A. (2004). Prokaryote diversity and taxonomy: current status and future challenges. *Philosophical Transactions of the Royal Society B: Biological Sciences* 359: 623-38.
[3] Whitman WB (2014). The Need for Change: Embracing the Genome. *Methods in Microbiology* 41: 1-12.

CHEMOTAXONOMY: FROM SAFETY TO WHERE?

Iain C. Sutcliffe*
Department of Applied Sciences, Northumbria University UK
Email: iain.sutcliffe@unn.ac.uk

Since the early 1970s, chemotaxonomy has played an important and integral role in the polyphasic approach to the taxonomy of prokaryotes. In particular, analysis of cell envelope components (membrane lipids, fatty acids, peptidoglycan and cell wall components etc) has been of considerable value. However, many of the methodological procedures routinely employed suffer from poor resolution, reproducibility and lack of operator expertise. As we enter the era of genome sequencing-based taxonomy, with attendant potential for "in silico chemotaxonomy", the need for the routine integration of chemotaxonomy into contemporary practise can and should be questioned.

OBJECTIVE TAXA DELINEATION WITH CANDIDATE TAXONOMIC UNIT CONCEPT

Pelin Yilmaz*
MPI for Marine Microbiology, Celsiusstr. 1, D-28359 Bremen, Germany
Email: pyilmaz@mpi-bremen.de

The number of environmental rRNA gene sequences has surpassed the number of sequences from cultivated microorganisms by far. Hence, reconciliation of the established taxonomy and a classification of the uncultured fraction have become critical. A major concern is that only lower rank categories are appropriately circumscribed by established rules and that the higher taxa circumscriptions are devoid of robust rules. Thus, there is an urgent need to establish objective criteria for higher ranks. Additionally, reconsidering the classification of uncultured Archaea and Bacteria is not covered by the Bacteriological Code, and only a "Candidatus" status can be given to those uncultured organisms, leaving no possibility to generate a unified nomenclature for both cultured and uncultured microorganisms. As the simultaneous existence of several nomenclatural systems does not impede building a common classification, we suggest that it is possible to have a stable and unified taxonomy that includes all Bacteria and Archaea, either culturable or not. In light of this, we pursued the question whether or not it is possible to define numerical thresholds of 16S rRNA gene sequence identity for higher taxa based on the established current taxonomy.

We first analyzed the sequence identities shared between higher taxa of Bacteria and Archaea and found that taxonomists had indeed applied quite homogeneous criteria that could form a robust framework for a coherent and stable classification. Based on the sequence identities we created thresholds which we subsequently applied to environmental sequences in order to understand the extent of diversity hitherto discovered. As a result of our analyses, we suggest a new methodology for the classification of candidate taxa as well as a nomenclature of putative bacterial and archaeal taxa. Additionally, with the new taxonomic thresholds, we estimated that at the current rate of sequencing, most of the taxa present in common habitats will be discovered by the end of the decade. We also concluded that almost complete sequences are necessary to accurately measure the taxonomic diversity.

NOCARDIOPSIS: FROM ITS BIODIVERSITY TO MECHANISM OF ENVIRONMENTAL ADAPTABILITY BASED ON BIOMICS METHODS

Wen-Jun Li[1,2]

[1] School of Life Sciences, Sun Yat-Sen University, Guangzhou, 510275, China; [2] Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Ürűmqi 830011, China

Email: liwenjun3@mail.sysu.edu.cn

The genus *Nocardiopsis*, which is a widespread group of the phylum *Actinobacteria*, has received great attention owing to its ecological versatility, pathogenicity and ability to produce a rich array of bioactive metabolites. Before the year of 2000, there are only seven validly published species in this genus. However, now it has expanded to 43 species and two subspecies. And recently, more and more strains of the genus *Nocardiopsis* have been isolated from saline soils or saline-alkali soils by our group and other colleagues in the world, and most of them being strictly halophilic.

To shed light on speciation, gene content evolution, and environmental adaptation in these unique actinobacteria, we sequenced draft genomes for 16 representative species of the genus and compared them with that of the type species *N. dassonvillei* subsp. *dassonvillei* DSM 43111^T. The core genome of 1,993 orthologous and paralogous gene clusters was identified, and the pan-genomic reservoir was found not only to accommodate more than 22,000 genes, but also to be open. The top ten paralogous genes in terms of copy number could be referred to three functional categories: transcription regulators, transporters, and synthases related to bioactive metabolites. Based on phylogenomic reconstruction, we inferred past evolutionary events, such as gene gains and losses, and identified a list of clade-specific genes implicated in environmental adaptation.

Moreover, we performed iTRAQ-based quantitative proteomics of *Nocardiopsis xinjiangensis* YIM 90004^T to investigate the function of the membrane proteome during salt stress. A total of 687 membrane proteins were identified and accurately quantified at the protein level, of which 131 membrane proteins displayed salt-induced changes in abundance, including 83 that were up-regulated and 53 that were down-regulated. Intriguingly, bioinformatics analyses indicated that these differentially expressed proteins could be separated into two typical protein expression patterns. The majority of ABC transporters, secondary active transporters, cell motility proteins, and signal transduction kinases were up-regulated with increasing salt concentration, whereas cell differentiation, small molecular transporters (ions and amino acids), and secondary metabolism proteins were significantly up-regulated at optimum salinity; however, they were down-regulated or relatively unchanged at higher salinity. More importantly, these two expression models were further validated with phenotypic changes and functional differences analysis. The small molecule transporters and cell differentiation proteins behaved as sensing proteins, which played a more important biological role at optimum salinity. However, the ABC transporters for compatible solutes, Na+-dependent transporters and cell motility proteins behaved as adaptive proteins that conspicuously and actively counteracted higher salinity stress. Overall, membrane proteins appear to be one of the major protection strategies against hyperosmotic stress. These results provided insights into the genetic causes of environmental adaptability in this cosmopolitan actinobacterial group and the contributions made by its inherent features, including genome dynamics and the constituents of core and accessory proteins.

References

[1] Yong-Guang Zhang, Xin-Hua Lu, Yan-Bo Ding, Xing-Kui Zhou, Hong-Fei Wang, Jian-Wei Guo, Yong-Hong Liu, Yan-Qing Duan, **Wen-Jun Li***. *Nocardiopsis rhizosphaerae* sp. nov., a new member of the genus *Nocardiopsis*, isolated from rhizosphere soil of *Halocnermum strobilaceum* (Pall.) Bieb. ***Int. J. Syst. Evol. Microbiol.*** 2016,

[2] Ming-Wei Sun, Xiao-Mei Zhang, Hui-Li Bi, **Wen-Jun Li*** & Chun-Hua Lu*. Two new sesquiterpenoids produced by halophilic *Nocardiopsis chromatogenes* YIM 90109. ***Natural Product Research***. http://dx.doi.org/10.1080/14786419.2016.1214831.

[3] Yao Zhang, Yanchang Li, Yongguang Zhang, Zhiqiang Wang, Mingzhi Zhao, Na Su, Tao Zhang, Lingsheng Chen, Wei Wei, Jing Luo, , Yanxia Zhou, Yongru Xu, Ping Xu*, **Wenjun Li*,** Yong Tao*. Quantitative Proteomics Reveals Membrane Protein-Mediated Hypersaline Sensitivity and Adaptation in Halophilic *Nocardiopsis xinjiangensis*. ***Journal of Proteome Research***. 2016 Jan 4;15(1):68-85. doi: 10.1021/acs.jproteome.5b00526

[4] Yong-Guang Zhang, Qing liu, Hong-Fei Wang, Dong-Jin Park, Jian-Wei Guo, Chang-Jin Kim, Yuan-Ming Zhang, Wen-Jun Li*. *Nocardiopsis ansamitocini* sp. nov., a new producer of ansamitocin P-3 of the genus *Nocardiopsis*. ***Int J Syst Evol Microbiol.*** 2016 Jan;66(1):230-235. doi: 10.1099/ijsem.0.000703.

[5] Hua-Qi Pan, Dao-Feng Zhang, Li Li, Zhao Jiang, Juan Cheng, Yong-Guang Zhang, Hong-Fei Wang, Jiang-Chun Hu, **Wen-Jun Li***. *Nocardiopsis oceani* sp. nov. and *Nocardiopsis nanhaiensis* sp. nov., two novel actinomycetes isolated from marine sediment of South China Sea. *Int. J. Syst. Evol. Microbiol.* 2015 Oct;65(10):3384-3391. doi: 10.1099/ijs.0.000425.

[6] Song-Tao He, Xiao-Yang Zhi, Hongchen Jiang, Ling-Ling Yang, Jin-Yuan Wu, Yong-Guang Zhang, Wael N.Hozzein & **Wen-Jun Li***. Biogeography of *Nocardiopsis* strains from hypersaline environments of Yunnan and Xinjiang Provinces, western China. ***Scientific Reports***. 2015, 5:13323. doi:10.1038/srep13323

[7] Mingwei Sun, Xiaomei Zhang, Huilin Hao, **Wenjun Li***, and Chunhua Lu*. Nocarbenzoxazoles A-G, Benzoxazoles Produced by Halophilic *Nocardiopsis lucentensis* DSM 44048. ***J Nat Prod***. **2015**, 78 (8), pp 2123–2127 **DOI:** 10.1021/acs.jnatprod.5b00031.

[8] Dao-Feng Zhang, Hua-Qi Pan, Jie He, Xiao-Mei Zhang, Yong-Guang Zhang, Hans-Peter Klenk, Jiang-Chun Hu & **Wen-Jun Li***. Description of *Streptomonospora sediminis* sp. nov. and *Streptomonospora nanhaiensis* sp. nov., and reclassification of *Nocardiopsis arabia* (Hozzein & Goodfellow, 2008) as *Streptomonospora arabica* comb. nov. and emended description of the genus *Streptomonospora*. ***Int. J. Syst. Evol. Microbiol.*** 2013, 63(12): 4447–4455;

[9] Hong-Wei Li, Xiao-Yang Zhi, Ji-Cheng Yao, Yu Zhou, Shu-Kun Tang, Hans-Peter Klenk, Jiao Zhao, **Wen-Jun Li*.** Comparative Genomic Analysis of the Genus *Nocardiopsis* Provides New Insights into its Genetic Mechanisms of Environmental Adaptability. ***PLoS ONE***. 2013, 8(4): e61528. doi:10.1371/journal.pone.0061528.

PROTEOTYPING: TANDEM MASS SPECTROMETRY PROTEOMICS AND WHOLE GENOME SEQUENCE-BASED DIAGNOSTICS OF INFECTIOUS BACTERIA IS DEPENDENT UPON A RELIABLE AND COMPREHENSIVE SYSTEMATIC FRAMEWORK

E.R.B. Moore[1,2]*, L. Gonzales Siles[1]. F. Salvà Serra[2,3], H. Engström Jakobsson[1], F. Boulund[4], E. Kristiansson[4], R. Karlsson[1,5]

[1] Department of Infectious Disease and [2] Culture Collection University of Gothenburg (CCUG), Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [3]Department of Biological Sciences, University of the Balearic Islands, Palma de Mallorca, Spain; [4] Department of Mathematical Statistics, Chalmers University of Technology, Gothenburg, Sweden; [5] Nanoxis Consulting AB, Gothenburg, Sweden

Email: erbmoore@ccug.se

The global expansion of anti-microbial resistance (AMR) in bacteria, including human pathogens, presents major challenges for treatment and preventing the spread of infection. **The World Health Organisation (WHO) has predicted the advent of infectious diseases for which no antibiotic treatment will be available** [1]. With this outlook in the escalation of AMR, combined with continuing decline in new antibiotic discovery, development of innovative, reliable, rapid and cost-efficient analytical techniques for effective diagnostics and characterisations of infectious microorganisms is increasingly essential to prevent rising mortality and to reduce the costs associated with antibiotic-resistant infections. However, the routine methodologies used today for diagnosing infectious bacteria depend upon protocols that require prior cultivation from samples. Faced with patients exhibiting symptoms of infection, physicians typically resort to prescribing broad-spectrum antibiotics while they wait days or weeks for results from the laboratory.

With increasing whole-genome DNA sequence data becoming available, MS-based proteomics also have increasingly been applied to biological studies. Proteomic analyses of bacterial cells may be considered indirect analyses of the genomes of bacteria. The 'proteome' comprises the entire set of proteins expressed by a cell, an organism or a biological system. 'Proteotyping' [2], using state-of-the-art LC-MS/MS analyses of generated cellular peptides, enables identification of the most closely related bacterial species and sub-species-level strain discrimination, AMR- and virulence-factors, from single MS analyses. Comprehensive and accurate genome sequence data is the key to obtaining accurate peptide matching and to be able to discriminate between the most closely related species. In this study, genome sequences were analysed, using Average Nucleotide Identity Blast (ANIb) and taxon-specific MLSA to assess their reliabilities. Critically, significant numbers of sequenced genomes in the public databases exhibited questionable identifications.

Characterisations and identifications of responsible agents of infectious disease have relied heavily upon established systematic frameworks and the documented features of well-described microbial species. As methodologies, such as whole-genome sequencing and MS proteomics are developed to enable more comprehensive, detailed and complex analyses, comprehensive databases derived from a reliable systematic framework are essential.

References

[1] World Health Organization (WHO). (2014) Antimicrobial Resistance: Global Report on Surveillance. ISBN: 978-92-4-15674-8.

[2] Karlsson et al. [2015) Proteotyping: Proteomic characterization, classification and identification of microorganisms – A prospectus. *Syst. Appl. Microbiol.* 38: 246-257.

METABOLOMICS IN BACTERIAL TAXONOMY

Ch. Sasikala[1]* and Ch. Venkata Ramana[2]
[1]Bacterial Discovery Laboratory, Centre for Environment, IST, JNT University Hyderabad, Hyderabad 500085, Telangana, India; [2]Department of Plant Sciences, University of Hyderabad, Hyderabad 500046, Telangana, India
Email: sasikala.ch@gmail.com; sasi449@yahoo.ie

Chemistry of small (<1000 Da) organic molecules plays a vital role in understanding a biological system since these are the intermediates or products of metabolism representing phenotypes and functional genomics of a biological system. For a taxonomist, small molecular chemistry (chemotaxonomy) plays pivotal role in the identification of taxa and helps in distinguishing taxa, particularly at the level of species or genus. Chemotaxonomy is traditionally restricted to comprise fatty acids, quinones/terpenoids, polyamines, polar lipids, cell-wall amino acids, carbohydrates, or secondary metabolites, but has sometimes been defined so broadly that it also includes DNA sequences. However, most of these metabolites are only independently looked at. From a holistic point of view, metabolomics provides an opportunity to cover all the metabolites originating from the entire metabolic machinery in an organism. The exo-metabolomes represent the foot-print of an organism, while the endo-metabolomes are the fingerprints. As on date, there is no one good protocol for the global analysis of metabolites. Most of the metabolome analyses are target based and greatly depend on the analytical platforms and post processing of the data using high end statistical methods of analysis. Our lab is mainly focusing on the lipidome and fermentome analysis for typing bacteria and as one of the taxonomic characters in distinguishing taxa, details of which will be discussed during presentation along with the concept of "*integrated taxonomy*".

THE LONG VOYAGE TO A GENOMIC ENCYCLOPAEDIA OF BACTERIA AND ARCHAEA: THE FIRST DECADE

M. Göker[1], J. Meier-Kolthoff[1], N. Shapiro[2], T. Woyke[2], N. C. Kyrpides[2,3] and H.-P. Klenk*[4]
[1] Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany; [2] DOE Joint Genome Institute, Walnut Creek, CA, USA; [3] Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jaddah, Saudi Arabia; [4] School of Biology, Newcastle University, Newcastle upon Tyne, UK
Email: hans-peter.klenk@ncl.ac.uk

The project to generate a Genomic Encyclopedia of *Bacteria* and *Archaea* (GEBA) was initiated almost one decade ago following a recommendation of an American Academy of Sciences colloquium on *Reconciling Microbial Systematics & Genomics* in Fall 2006. After a successful pilot project run as collaboration between the DOE Joint Genome Institute (JGI) and the German Collection of Microorganisms and Cell Cultures (DSMZ) [1] proved the value of the phylogeny-driven initial approach for phylogeny, taxonomy, description of novel species, but also the binning of metagenomics studies, the project was extended by several large scale sequencing projects [2].
The first of these one thousand genome sequences (each) strong follow-up projects [3] reached now the publication phase, while phase II (from individual species to whole genera) and phase III (the genomes of soil and plant-associated and newly described type strains [4]) are deep into the genome sequence production stage. GEBA is meanwhile complemented by similarly structured spin-offs, such as the 'One thousand Actinomycetes' project run by the same culture collections and sequencing centre. Results and impact of these large scale projects on the description of novel taxa shall be reported.

References

[1] Wu, D,, Hugenholtz, P., Mavromatis, K., Pukall, **…** [26 authors] **…** Kyrpides, N.C., Klenk, H.P., Eisen, J.A. (2009) A phylogeny-driven genomic encyclopaedia of *Bacteria* and *Archaea*. *Nature* 462: 1056-1060.

[2] Kyrpides, N.C., Hugenholtz, P., Eisen, J.A., Woyke, T., Göker, M. … [56 authors] … Whitman, W.B., Garrity, G.M., Klenk, H.P. (2014) Genomic Encyclopedia of *Bacteria* and *Archaea*: sequencing a myriad of type strains. *PLoS Biology* 12: e1001920.

[3] Kyrpides, N.C., Woyke, T., Eisen, J.A., Garrity, G.M., Lilburn, T.G., Beck, B.J., Whitman, W.B., Hugenholtz, P., Klenk, H.P. (2014) Genomic Encyclopedia of type strains, phase I: the one thousand microbial genomes (KMG-I) project. *Stand Genomic Sci* 9: 1278-1284.

[4] Whitman, W.B., Woyke, T., Klenk, H.P., Zhou, Y., Lilburn, T.G., Beck, B.J., De Vos, P., Vandamme, P. Eisen, J.A., Garrity, G.A., Hugenholtz, P., Kyrpides, N.C. (2015) Genomic Encyclopedia of bacterial and archaeal type strains, phase III: the genomes of soil and plant-associated and newly described tpye strains. *Stand Genomic Sci* 10: 26.

Session 5 – Minimum Standards for the Description of New Taxa

GENEALOGICAL CONCORDANCE AND OTHER LINES OF EVIDENCE FOR THE RECOGNITION AND DESCRIPTION OF BACTERIAL SPECIES

S.N. Venter*, W.Y. Chan, M. Palmer, G. Shin, C.W. Beukes, E van Zyl, T.A. Coutinho and E.T. Steenkamp

Department of Microbiology and Plant Pathology and DST-NRF Centre of Excellence in Tree Health Biotechnology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa

Email: fanus.venter@up.ac.za

Bacterial species are commonly defined by applying a set of predetermined criteria, including DNA:DNA hybridization values, 16S rRNA sequence similarity and phenotypic and chemotaxonomic comparisons. Alternative genome-based similarity criteria have also been proposed to define bacterial taxa. These criteria mostly allow for the delimitation of taxa that resemble typical bacterial species. Their application is, however, often complicated when the objective is to delineate new species that are characterized by significant population-level diversity or that evolved early. This is because most of the predetermined taxonomic criteria utilize species cut-off values that are often not appropriate for older or more diverse taxa. However, we believe that these complexities and limitations can be easily circumvented by recognizing that bacterial species represent unique and exclusive assemblages of diversity. Within such a framework, methods that account for the population processes involved in species evolution are used to infer species boundaries, while the resulting species hypothesis are tested using additional biological data. In practice, a method such as genealogical concordance analysis allows for the generation of species hypotheses (i.e. this method delineates a putative species). The existence of the new taxon is then interrogated using an array of characters that include the traditionally used criteria, as well as genome-based criteria such as gene content and average nucleotide identity (ANI). By making use of taxa in the genera *Escherichia, Pantoea* and *Paraburkholderia* we demonstrate how genealogical concordance can be used to delimit a bacterial taxon. Other biological criteria were used to provide independent lines of evidence for the existence of that taxon. This approach to species recognition and description is straightforward and applicable to bacterial species of all ages and diversities. It is particularly useful in the post-genomic era, with whole genome sequences now being accessible for taxa of interest. In fact, our results indicated that a combined genome-based comparative and evolutionary approach would be the preferred alternative for delineating coherent bacterial taxa.

NEXT GENERATION BACTERIAL GENOME ASSEMBLY AND METAGENOMES

Stephen Rudd*
Pacific Biosciences, 1380 Willow Road Menlo Park, CA 94025, USA

Single molecule real-time (SMRT) DNA sequencing underpins the third generation of sequencing technology. Sequencing libraries are created from sheared and size-selected genomic DNA – current protocols accommodate fragments of over 30kb in size. Millions of sequencing reactions are performed in parallel within a Zero Mode Waveguide - each containing a single molecule of DNA polymerase and its template DNA. The incorporation of phospholinked nucleotides is captured as a continuous and native process. The characteristics of SMRT sequencing are reflected as reads of varying length with a mean read length of 12kb and with a subset of the long reads that extend to over 50kb in length.

This presentation reviews the application of long DNA sequence reads in today's biological and clinical research. Long DNA sequences have already changed our ability to assemble bacterial and eukaryotic genomes – many species now have high quality PacBio enabled genome assemblies with a contig N50 of megabases in length.

The application of long sequence reads is not limited to genome assembly and case studies will be presented that demonstrate

1. Amplicon sequences from the 16S rRNA can be used with the greatest sensitivity to profile the species-level diversity across clinical and environmental microbial samples
2. "Shotgun" sequencing from complex microbial communities can be used to recover complete genome sequences and large contiguous genomic blocks from less represented species – the genomic context enables comparative functional genomics
3. PacBio has become the gold standard in the de novo genome assembly of microbial and eukaryotic genomes. Examples of sequenced genomes that cover the tree of life will be presented
4. The kinetic data associated with the long read sequencing can also be used in the discovery and characterization of DNA base modifications – this provides both the genome and its epigenomic landmarks in a single process without changes or modifications to protocol.

Session 6 – Cyanobacterial Taxonomy

A SHARED SPECIES DEFINITION IS NEEDED FOR A UNIFIED APPROACH TO THE TAXONOMY OF CYANOBACTERIA

S. Ventura*
National Research Council of Italy, Institute of Ecosystem Study, Sesto Fiorentino, Italy
Email: stefano.ventura@ise.cnr.it

To classify all living organisms, a binomial system having the species as the fundamental taxonomic unit is in use for several centuries. This system is based on the species concept and definition, but while the species concept remains, or should remain the same, the species definition requires to be adapted to the features of each group of organisms. This is also reflected in the development of different taxonomic practices, standards, and rule sets for each kingdom or domain.

Historically, cyanobacterial taxonomy developed in the botanical world because Cyanobacteria have been ever since identified as algae, but they are true prokaryotes and in more recent times a claim has been raised to include them in the bacterial taxonomic system. This poses nomenclature issues difficult to solve and an attempt to harmonize their treatment under two Codes is presently not progressing.

A possible solution could be a definition of the cyanobacterial species shared by the botanical and bacteriological communities. A general agreement on the basic features of the cyanobacterial species would contribute to build common judgement and scientific acceptance to old and new taxa of Cyanobacteria, paving the way for the assembling of an approved list of cyanobacterial species and for the future development of a unified approach to cyanobacterial taxonomy.

The more critical aspects for the definition of the cyanobacterial species will be presented, including the use of morphometric data and variable morphological traits coupled with complex life cycles; the unclear distinction between species with restricted habitat and local natural population; the amount of infra-specific diversity.

NEW SPECIES OF NOSTOC (CYANOBACTERIA) ISOLATED FROM PUNE, INDIA USING MORPHOLOGICAL, ECOLOGICAL AND MOLECULAR ATTRIBUTES

P. Singh[1]*, Z.M. Shaikh[1], L.A. Gaysina[2], A. Suradkar[1], U. Samanta[1]
[1]*Microbial Culture Collection, National Centre for Cell Science, India*
[2]*Department of Bioecology and Biological Education, M. Akmullah Bashkir State Pedagogical University, 450000 Ufa, Okt'yabrskoi revolucii 3a, Russian Federation*
Email: sps.bhu@gmail.com

Filamentous cyanobacterium (strain NE-PS) isolated from a fresh water body in Pune, India is being described as new species of the polyphyletic genus *Nostoc*. Phenotypic and molecular characterizations were performed and the combined results validated the strain as a new species. Careful observations of the filaments, presence of a distinct sheath throughout the length of the trichome, prominent differences in the shape and dimensions of the vegetative cells, heterocytes and the akinetes provided reliable morphological signals that the strain differed from rest of the closely related species. Sequencing of the 16S rRNA gene showed 98.66% sequence similarity with *Nostoc linckia* while *rbcl* and *psb*A sequencing showed 97% and 94% similarities with *Nostoc* sp. PCC 7906 and *Nostoc punctiforme* PCC 73102 respectively while the *nif*D gene sequence similarity was found to be 96% with *Nostoc punctiforme* Ind35 and *Desmonostoc muscorum*. The PC-IGS region was sequenced and concatenated *cpc*B, IGS and *cpc*A regions indicated the closest similarity with *Nostoc linckia* PACC 5085 at 96%. Subsequent phylogenetic analyses gave a strong pattern of distinct clustering in case of all the molecular markers. The phenotypic, genetic and phylogenetic observations prove conclusively that the strain NE-PS is a new species in the genus *Nostoc* with the name proposed being *Nostoc punensis*, sp. nov.

Poster Abstracts

BISMiS-2016 International Student Travel Award Recipient

DEVELOPMENT OF GENOMIC TOOLS TO PREDICT POLAR LIPID PRODUCTION

N. B. Patel,*[1] K. Sankaranarayanan,[2] P. A. Lawson[1]
[1]*Department of Microbiology & Plant Biology;* [2]*Department of Anthropology, University of Oklahoma, Norman, Oklahoma, USA*
Email: npatel@ou.edu

The characterization of novel microorganisms traditionally requires a polyphasic approach that includes phylogenetic, biochemical and chemotaxonomic investigations. Analytical methods for characterizing chemotaxonomic biomarkers are typically labor intensive and expensive. Even with the repeated use of reference strains there is a lack of curated databases and standardized protocols, makes reproducibility of these tests and comparative analysis of results difficult. Alternatively, genomic information, specifically the identification of genes involved in metabolic pathways associated with the biosynthesis of these important taxonomic markers. can serve as a powerful tool for more rapid identification and classification. We hypothesized that this "*in silico*" approach to chemotaxonomy can complement existing laboratory protocols, to aid in the for more rapid identification and classification of microorganisms. We evaluated this approach by first using organisms from five dominant phyla to compare TLC analysis against genomic predictions. Several trends were identified between these data sets ranging from complete concordance (a match between TLC and genome) for the polar lipid Phosphatidylethanolamine, to phylum specific concordance as observed for the polar lipid Diphosphatidylglycerol among *Firmicutes*. Collectively, these results highlight several challenges currently facing "*in silico*" approaches to chemotaxonomy. These include (a) the need for experimental characterization of biosynthesis pathways in a phylogenetically diverse range of organisms, and, (b) standardized reporting for "*in vitro*" characterizations of taxonomic markers. Further analysis will continue to expand the current number of organisms examined to determine additional patterns. Addressing these challenges will allow for more refined hypothesis testing, and generation of phylogenetically specific sequence models and curated databases for "*in silico*" markers, providing reliable chemotaxonomic information that is accessible to the larger scientific community.

ANALYSIS OF TRUE TRNA DIVERSITY AMONG UNCULTURED ARCHAEA AND BACTERIA

Bhagwan N. Rekadwad* and Chandrahasya N. Khobragade
School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded 431606, India.
E mail: rekadwad@gmail.com

Complete genome sequences of two uncultured archaea (BX649197 & CR937008) and 10 uncultured bacteria (AC160099, FP245538-FP245540, FP312972, FP312974-75, FP312977, FP312985 & NZ_JPJG01000067) were downloaded from nucleotide database and chosen for identification and study of tRNA diversity. tRNAscan-SE and ENDMEMO GC calculator freeware were used for detection of tRNA, drawing structures and calculation of GC percent. Seven archaeal and 48 bacterial tRNA were detected from above 12 sequences. Of these, four archaeal and 30 bacterial tRNA showed cove score more than 20% are called as true tRNA. Three tRNA of uncultured bacteria (AC160099) has the presence of the variable loop. The tRNA of FP245540, FP245575, FP245577 & FP245585 has one variable loop each. The true tRNA of archaea was classified as Alanine, Arginine & Cysteine-type tRNA, while the majority of bacteria true tRNA classified as Alanine, Glutamic acid, Isoleucine, Leucine, Methionine, Phenylalanine, Proline and Valine-type tRNA with cove score ranged from 70%-97.15%. Complete genome sequences of archaea and bacteria have GC content approximately 43% and 34.7%-63.3% respectively. Archaeal tRNA has 60.4%-64.2% GC content. Similarly, bacterial tRNA contributed 49.3%-66.3% GC content, which increased total GC percentage of uncultured microorganisms. Thus, it is concluded that selected uncultured archaea and bacteria has the diversity of true tRNA. The reason presence of tRNA diversity may be selective mechanism imposed by the growth temperature on the evolution tRNA diversity. Also, the pairing between 'G' and 'C' is stronger than between 'G' and 'U', which might be the reason for the presence of higher tRNA diversity.

References

[1] Targanski, I. and Cherkasova, V. (2008) Analysis of genomic tRNA sets from Bacteria, Archaea, and Eukarya points to anticodon-codon hydrogen bonds as a major determinant of tRNA compositional variations. RNA 14:1095-1109.

[2] Silva, F. J., Belda, E. and Talens, S. E. (2006) Differential annotation of tRNA genes with anticodon CAT in bacterial genomes. Nucleic Acids Research 34:6015-6022.

[3] Lowe T. M., and Eddy, S. R. (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Research 25:955-964.

[4] Siddhartha Satapathy, S., Dutta M. and Ray, S. K. (2010) Variable correlation of genome GC% with transfer RNA number as well as with transfer RNA diversity among bacterial groups: a-Proteobacteria and Tenericutes exhibit strong positive correlation. Microbiological Research 165:232-242.

PHYLOGENOMICS REVEAL HIDDEN TAXONOMIC DIVERSITY IN CLINICAL ISOLATES OF *STENOTROPHOMONAS MALTOPHILIA*

Prashant P. Patil*[1], Samriti Midha*[1], Sanjeet Kumar*[1], Vikas Gautam*[2] and Prabhu B. Patil*[1]
[1]*Laboratory of Bacterial Genomics and Evolution, CSIR-Institute of Microbial Technology, Chandigarh;*
[2]*Department of Medical Microbiology, Post Graduate Institute of Medical Education and Research, Chandigarh.*
Email: prashantpatil@imtech.res.in

Stenotrophomonas maltophilia is an intrinsically drug resistant opportunistic human pathogen [1,2]. Phylogenomics of the type strains and historically important strains of has revealed at least 18 distinct species in genus *Stenotrophomonas* including one species of clinical origin apart from *S. maltophilia* [3]. Hence, we extended this study by sequencing whole genome of the 27 clinical isolates of *S. maltophilia* from diverse origin like blood, pus, cerebrospinal fluid, and respiratory specimens from the patients admitted to the tertiary care hospital of Post Graduate Institute of Medical Educations and Research (PGIMER), Chandigarh. Phylogenetic and taxonomic relationship of the clinical isolates was established using phylogenomic marker genes, Average Nucleotide Identity (ANI) and digital DNA-DNA hybridization (dDDH). Based on the cut-off values of ANI and dDDH for species delineation, there are six distinct genomospecies in the clinical isolates identified as *S. maltophilia*. Interestingly only 6 out of 27 isolates belongs to the *S. maltophilia* and remaining belong to at least five novel species. The study has revealed hidden taxonomic diversity in clinical isolates identified as *S. maltophilia*. Further studies supplemented with the conventional polyphasic approach are underway to ascertain these genomospecies as novel species.

References

[1] Ryan, Robert P., et al. "The versatility and adaptation of bacteria from the genus *Stenotrophomonas*." *Nature Reviews Microbiology* 7.7 (2009): 514-525.
[2] Looney, W. John, Masashi Narita, and Kathrin Mühlemann. "*Stenotrophomonas maltophilia*: an emerging opportunist human pathogen. "*The Lancet infectious diseases* 9.5 (2009): 312-323.
[3] Patil, P. P., Midha, S., Kumar, S., & Patil, P. B. (2016). Genome sequence of type strains of genus *Stenotrophomonas*. *Frontiers in Microbiology*, *7*.

DIVERSITY OF CULTURABLE THERMOPHILIC ACTINOBACTERIA IN HOT SPRINGS IN TENGCHONG, CHINA AND STUDIES OF THEIR BIOSYNTHETIC GENE PROFILES

Lan Liu*[1], Nimaichand Salam[1], Jian-Yu Jiao[2,3], Hong-Chen Jiang[4], En-Min Zhou[1,2], Yi-Rui Yin[2], Hong Ming[2,5], Wen-Jun Li[1,2]

[1] State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-Sen University, Guangzhou, China; [2] Yunnan Institute of Microbiology, Yunnan University, Kunming, China; [3] Medical Faculty of Kunming University of Science and Technology, Kunming University of Science and Technology, Kunming, China; [4] State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan, China; [5] College of Life Sciences and Technology, Xinxiang Medical University, Xinxiang, China

Email : *liwenjun3@mail.sysu.edu.cn* *jiangh@cug.edu.cn*

The class *Actinobacteria* has been a goldmine for the discovery of antibiotics and has attracted interest from both academics and industries. However, an absence of novel approaches during the last few decades has limited the discovery of new microbial natural products useful for industries. Scientists are now focusing on the ecological aspects of diverse environments including unexplored or underexplored habitats and extreme environments in the search for new metabolites. This paper reports on the diversity of culturable actinobacteria associated with hot springs located in Tengchong County, Yunnan Province, southwestern China. A total of 58 thermophilic actinobacterial strains were isolated from the samples collected from ten hot springs distributed over three geothermal fields (e.g., Hehua, Rehai, and Ruidian). Phylogenetic positions and their biosynthetic profiles were analyzed by sequencing 16S rRNA gene and three biosynthetic gene clusters (KS domain of PKS-I, KSα domain of PKS-II and A domain of NRPS). On the basis of 16S rRNA gene phylogenetic analysis, the 58 strains were affiliated with 12 actinobacterial genera: *Actinomadura*, *Micromonospora*, *Microbispora*, *Micrococcus*, *Nocardiopsis*, *Nonomuraea*, *Promicromonospora*, *Pseudonocardia*, *Streptomyces*, *Thermoactinospora*, *Thermocatellispora*, and *Verrucosispora*, of which the two novel genera *Thermoactinospora* and *Thermocatellisopora* were recently described from among these strains. Considering the biosynthetic potential of these actinobacterial strains, 22 were positive for PCR amplification of at least one of the three biosynthetic gene clusters (PKS-I, PKS-II, and NRPS). These actinobacteria were further subjected to antimicrobial assay against five opportunistic human pathogens (*Acinetobacter baumannii*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus* and *Streptococcus faecalis*). All of the 22 strains that were positive for PCR amplification of at least one of the biosynthetic gene domains exhibited antimicrobial activities against at least one of the five test organisms. Among the remaining 36 actinobacteria that are negative for PCR amplification of the domains for the biosynthetic genes, 33 strains showed antimicrobial activities against at least one of the five test pathogens. In summary, the findings presented in this study emphasized the importance of underexplored habitats such as Tengchong hot springs as potential sources for search of bioactive molecules.

Reference

[1] Lan Liu, Nimaichand Salam, Jian-Yu Jiao, Hong-Chen Jiang, En-Min Zhou, Yi-Rui Yin, Hong Ming, Wen-Jun Li. Diversity of culturable thermophilic actinobacteria in hot springs in

Tengchong, China and studies of their biosynthetic gene profiles. *Microbial Ecology*. 2016, DOI: 10.1007/s00248-016-0756-2

DIVERSITY OF SYMBIOTIC BACTERIA ASSOCIATED WITH PUFFER FISH *GASTROPHYSUS SPADICEUS* AND THEIR ANTIBACTERIAL ACTIVITIES

Yi-Huan Wang[1]*, Qing Liu[1,2], Zi-Wen Yang[1], Nimaichand Salam[1], Li-Xiang Cao[1], Min Xiao[1], Jian-Hua Ju[2] and Wen-Jun Li[1*]

[1] State Key Laboratory of Biocontrol and Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-Sen University, Guangzhou 510275, China; [2] CAS Key Laboratory of Tropical Marine Bio-resources and Ecology, Guangdong Key Laboratory of Marine Materia Medica, RNAM Center for Marine Microbiology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

Email: liwenjun3@mail.sysu.edu.cn; wangyih6@mail2.sysu.edu.cn

Tetrodotoxin (TTX) is a neurotoxin produced by many marine bacteria and often bioconcentrated along the food chain including several species of puffer fish [1]. Human cases of TTX poisoning from consumption of such fishes resulting in paresthesias, ataxia, diarrhea, vomiting, respiratory insufficiency, paralysis and even death have been reported [2]. In a toxicity assessment of the common puffer fish species in South China Sea, the species *Gastrophysus spadiceus* was found to have low TTX content (TTX<10MU/g) and was therefore considered safe for consumption [3]. Till date, no systematic study has been conducted to assess the symbiotic bacterial diversity associated with the fish. The present study reports the diversity of culturable bacteria associated with the puffer fish *G. spadiceus*. We assumed that any symbiotic bacteria within the puffer fish are likely to exhibit interesting metabolic pathways that counteract TTX-toxicity. During the study, a total of 31 strains affiliated to the genera *Pseudomonas*, *Janthinobacterium*, *Rahnella, Psychrobacter* and *Yersinia* were isolated from liver, intestines and flesh of *G. spadiceus*. The metabolic potentials of the fermentation crude extracts of the strains were analyzed by HPLC and TLC. It was found that the strains exhibited a diverse range of metabolites. Some of these crude extracts showed strong antimicrobial activities against pathogenic bacterial strains. In addition, few crude extract exhibit insecticidal activity against *Artemia salina* L. It will be interesting to expand the studies revealed by these preliminary studies to give an insight about the biotechnological potential of these symbiotic bacteria with the use of combinatorial molecular tools involving both genomic and proteomics analyses.

References

[1] Mosher, H.S., Fuhrman, F.A., Buchwald, H.D. and Fisher, H.G. (1964). Tarichatoxin-Tetrodotoxin: a potent neurotoxin. *Science* 144: 1100-1110.

[2] Narahashi, T. (2001) Pharmacology of tetrodotoxin. *J Toxicology-Toxin Reviews* 20: 67-84.

[3] Liu, Z.Y. (2011) Study on common species and toxicity of Fugu from the South China Sea. *Journal of Fujian Fishes* 33: 40-44.

DIVERSITY OF HALOALKALIPHILIC BACTERIA FROM THE SALINE COASTAL DESERT OF LITTLE RANN OF KUTCH, GUJARAT (INDIA)

H. Bhatt* and S. Singh
UGC-CAS Department of Biosciences, Saurashtra University, Gujarat, India -360005
Email: satyapsingh@yahoo.com ; hitarth_bhatt@yahoo.co.in

In this report we describe bacterial diversity of yet unexplored, saline and enigmatic desert of Little Rann of Kutch. Being a coastal desert, it has a unique demography from the rest of the deserts in the world.Therefore, microbial studiesof this unique habitat may aid to prevent the spread of deserts, to restore soil/vegetation cover, to understand their ecological significance and explore their biotechnological potential. The haloalkaliphilic bacteria were isolated by direct plating and enrichment techniques. Five different media were used for the isolation to trap maximum cultivable diverse bacteria. The isolates were studied for the media characterization, morphological features, biochemical properties, pH tolerance, salt tolerance and antibiotic sensitivity profiles. The organisms were further studied for the secretion of extracellular enzymes and antagonistic properties. 16S r-DNA analysis revealed huge diversity displaying 2 different phylum; Firmicutes and Actinobacteria, 3 different families; *Bacillaceae, Planococcaceae and Micrococcaceae*, 9 different genera; *Bacillus, Halobacillus, Oceanobacillus, Virgibacillus, Salimicrobium, Bhargavaea, Alkalibacillus, Gracillibacillus and Micrococcus* and overall 20 different species. Phenotypic characters were used for the cluster analysis to group these bacteria into phenons using Jaccard similarity coefficient and UPGMA algorithm [1]. Diversity was judged by biphasic approach where genotypic and phenotypic profiles were taken into account. Phenotypic diversity was compared with genotypic diversity by comparing phenogram and phylogram where some of the isolates had unique phenotypic pattern and contradicted their phylogenetic placement. Species richness and evenness were determined by the diversity indices. Many of the isolates secreted multiple enzymes at high salinity and alkalinity indicating their biotechnological potential [2]. The study over all indicated that the Little Rann of Kutch could represent new lineages of the microorganisms providing unique source of enzymes and other valuable metabolites.

References

[1] Bhatt, H.B. and Singh, S.P. (2016) Phylogenetic and Phenogram based diversity of haloalkaliphilic bacteria from the saline desert. *Microbial Biotechnology: Technological challenges and developmental trends.* Apple Academic Press (Distribution by CRC press), New Jersey, USA.

[2] Raval, V.H., Rawal, C.M., Pandey, S., Bhatt, H.B., Dahima, B.R. and Singh, S.P. (2014) Cloning, heterologous expression and structural characterization of an alkaline serine protease from sea water haloalkaliphilic bacterium. *Annals of Microbiology* 65(1):371-381

DISTRIBUTION OF *MICROMONOSPORA SAELICESENSIS* IN LEGUMES

P. Benito*, J. De Fuentes, M. A. Infante and M. E. Trujillo
Departamento de Microbiología y Genética, Edificio Departamental, Campus Miguel de Unamuno, Universidad de Salamanca, Salamanca, Spain.
Email: patriciabs@usal.es

The genus *Micromonospora* in the family *Micromonosporaceae*, belongs to the phylum *Actinobacteria.* These bacteria are commonly found in different habitats which include soil, freshwater and marine sediments, and mangrove soil [1,2] In recent years, Micromonosporae strains have been reported to be present in root nodules of both leguminous and actinorhizal plants [3]. In the present study we report the presence of *Micromonospora saelicesensis* in other parts of the plant. The legume species *Pisum sativum* and *Lupinus angustifolius* were used for the isolation, they have been recovered, in addition to the nodules, from the leaves, roots and stems of the plants.

More than 150 *Micromonospora* morphology-like colonies were isolated of which 148 were confirmed to be *Micromonospora* strains. In total, 26 different species of *Micromonospora* were obtained, being *M. saelicesensis* the most abundant. The potential of these isolates for the degradation of plant polymers was evaluated by the production of hydrolytic enzymes such cellulases, xylanases, chitinases, amylases and pectinases. All *Micromonospora* isolates showed very high *in vitro* activities for cellulases and xylanases, while the number of strains with pectinase, amylase and chitinase activities was only detected in some strains. The isolates recovered from nodules showed the highest enzymatic activities, being the strains identified as *M. saelicesensis* the most active.

References

[1] Genilloud, O. (2012) "Genus *Micromonospora*," in *Bergey's Manual of Systematic Bacteriology*, Vol. 5, eds M. Goodfellow, P. Kämpfer, H.-J. Busse, M. E. Trujillo, K.-I. Suzuki, W Ludwig et al. (*New York, NY: Springer*), 1039–1057.
[2] Trujillo, M. E., Hong, K. and Genilloud, O. (2014) "Family Micromonosporaceae," in *The Prokaryotes, The Actinobacteria*, eds E. Ronsenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson (*Heidelberg: Springer*), 499–569.
[3] Carro, L., Pujic, P., Trujillo, M.E. and Normand, P. (2013) *Micromonospora* is a normal inhabitant of actinorhizal nodules. *Journal of Biosciences* 38: 685–693.

DEFINING THE SPECIES *MICROMONOSPORA SAELICESENSIS*

R. Riesco*, M. E. Trujillo, C. Prieto
Departamento de Microbiología y Genética, Edificio Departamental, Universidad de Salamanca, Salamanca, Spain.
Email: shot89_1000@usal.es

The species *Micromonospora saeliecesis* is a Gram-positive actinobacterium that was first isolated from nitrogen fixing nodules of the legume *Lupinus anustifolius* [1]. Continuing efforts to determine the distribution of *Micromonosporae* in legumes have confirmed the presence of this genus in all legumes sampled so far [2], being *M. saelicesensis* and *M. lupini* the most abundant respectively.

The recent genome sequence of the model strain *Micromonospora lupini* Lupac 08 highlighted several genomic features which may be important and has provided useful information as to how this bacterium may relate with its host plant. The genome data also revealed the potential of *M.lupini* Lupac 08 as a plant growth promoting bacterium [3].

In the present study we sequenced several strains identified as *Micromonospora saelicesensis* to determine the genetic diversity of the species in order to contribute to define the core and pangenomes of *Micromonospora saelicesensis*. These results were compared with the current standards to define a bacterial species (e.g. 16s rRNA gene sequence, MLSA and DDH).

References

[1] Trujillo M, Kroppenstedt R, Fernández-Molinero C, Schumann P, Martínez-Molina E. *Micromonospora lupini sp. nov. and Micromonospora saelicesensis sp. nov., isolated from root nodules of Lupinus angustifolius. International Journal Systematic Evolutionary Microbiology* 57(12):2799-2804

[2] Trujillo ME, Bacigalupe R, Pujic P, Igarashi Y, Benito P, et al. (2014) *Genome Features of the Endophytic Actinobacterium Micromonospora lupini Strain Lupac 08: On the Process of Adaptation to an Endophytic Life Style? PLOS ONE* 9(9): e108522.

[3] Trujillo ME, Alonso-Vega P, Rodríguez R, Carro L, Cerda E, Alonso P, Martínez-Molina E. (2010) *The genus Micromonospora is widespread in legume root nodules: the example of Lupinus angustifolius. ISME Journal* 4(10):1265-81

ISOLATION OF BACTERIORHODOPSIN PRODUCING HALOARCHAEON, *HALOSTAGNICOLA LARSENII* IBS (MCC 2956), FROM INDIAN BLACK SALT

P. P. Kanekar[1], S.O. Kulkarni[1]*, Y.Shouche[2], K. Jani[2], A.Sharma[2]
[1]Department of Biotechnology, Modern College of Arts, Science and Commerce, Shivajinagar, Pune 411005, M.S., India; [2]Microbial Culture Collection (MCC), National Centre for Cell Science (NCCS), Ganeshkhind, Pune 411007, M. S., India
Email: kanekarpp@gmail.com

Halophilic archaea are found distributed all over the world in natural saline environments like solar salterns, salt lakes, ancient salt sediments, rock salt and rarely from sea water [1]. Rock salt has been looked upon as a source of halophilic microorganisms by researchers over the globe. Haloarchaeal strains of *Haloarcula, Halorubrum*, *Halococcus*, *Halobacterium*, *Halolamina etc.* have been reported from ancient rock salt and salt deposits. Black Salt is a type of rock salt, used as a condiment in South Asia and a cooling spice in Indian Ayurvedic aid. A haloarchaeal strain was isolated from commercial Indian black salt using Sehgal and Gibbon's medium with 3.42 M NaCl and identified as *Halostagnicola larsenii* IBS by phenotypic characterization and 16 S rRNA gene sequencing. A few haloarchaeal species have been reported to produce bacteriorhodopsin (BR) [2], which acts as a light driven proton pump and converts light energy into electrical energy. The isolate IBS produced 0.288 g l^{-1} BR and the liquid culture of the isolate when exposed to sunlight for 8 hrs converted light energy into electric current of 12 mV. The present investigation appears to be the first report of isolation of *Halostagnicola larsenii* IBS (MCC 2956) from commercial Indian black salt.

Acknowledgement: The research work was financially supported by Council of Scientific and Industrial Research (CSIR), Govt. of India. The authors thank the Principal, Modern College of Arts, Science and Commerce, Shivajinagar, Pune for providing necessary facilities to carry out this work.

References

[1] Das Sarma S, Arora P (2001) Halophiles. *In Encyclopedia of Life Sciences*. pp. 1-9. Nature Publishing Group. www/els.net
[2] Oren A, Shilo M (1981) Bacteriorhodopsin in a bloom of halobacteria in the Dead Sea. *Achieves of Microbiology,* **130**: 185-187.

TAPPING THE UNTAPPED MICROBES: ACTINOBACTERIAL DIVERSITY IN LIMESTONE HABITAT

Nimaichand Salam[1,2]*, Mipeshwaree Devi Asem[1], Bao-Zhu Fang[1], Lan Liu[1], Bing-Bing Liu[1], En-Min Zhou[1], Debananda S. Ningthoujam[2] and Wen-Jun Li[1]

[1]State Key Laboratory of Biocontrol and Guangdong Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-Sen University, Guangzhou, 510275, China; [2]Microbial Biotechnology Research Laboratory, Department of Biochemistry, Manipur University, Canchipur, 795003, India

Email: s.nimaichand@hotmail.com

Studies on actinobacterial diversity in limestone habitats are scarce [1]. This paper reports profiling of actinobacteria isolated from Hundung limestone samples in Manipur, India using ARDRA as the molecular tool for preliminary classification. A total of 137 actinobacteria were clustered into 31 phylotypic groups based on the ARDRA pattern generated and representative of each group was subjected to 16S rRNA gene sequencing. Generic diversity of the limestone isolates consisted of *Streptomyces* (15 phylotypic groups), *Micromonospora* (4), *Amycolatopsis* (3), *Arthrobacter* (3), *Kitasatospora* (2), *Janibacter* (1), *Nocardia* (1), *Pseudonocardia* (1) and *Rhodococcus* (1). Of these, six have been characterized as novel strains of the genera *Streptomyces*, *Rhodococcus* and *Micromonospora*. [2-7]. In addition to the characterized species, another strains belonging to the genera *Amycolatopsis, Kitasatospora* and *Nocardia* are being characterized by polyphasic taxonomic approaches. Considering the antimicrobial potential of these actinobacteria, 86% of the strains isolated from Hundung limestone deposit sites possessed biosynthetic gene clusters of which 40% exhibited antimicrobial activities. It can, therefore, be concluded that limestone habitat is a promising source for search of novel secondary metabolites

References

[1] Nimaichand S, Devi AM, Tamreihao K, Ningthoujam DS, Li WJ (2015) Actinobacterial diversity in limestone deposit sites in Hundung, Manipur (India) and their antimicrobial activities. *Front Microbiol*, 6: 574 doi: 10.3389/fmicb.2015.00574

[2] Li WJ, Nimaichand S, Jiang Z, Liu MJ, Khieu TN, Kim CJ, Hozzein WN, Park DJ, Wadaan MAM, Ningthoujam DS (2014) *Streptomyces canchipurensis* sp. nov., isolated from a limestone habitat. *Antonie van Leeuwenhoek*, 106: 1119-1126

[3] Nimaichand S, Zhang YG, Cheng J, Li L, Zhang DF, Zhou EM, Dong L, Ningthoujam DS, Li WJ (2013) *Micromonospora kangleipakensis* sp. nov., isolated from a sample of limestone quarry. *Int J Syst Evol Microbiol*, 63: 4546-4551

[4] Ningthoujam DS, Nimaichand S, Ningombam D, Tamreihao K, Li L, Zhang YG, Cheng J, Liu MJ, Li WJ (2013) *Streptomyces muensis* sp. nov. *Antonie van Leeuwenhoek*, 104: 1135-1141

[5] Nimaichand S, Tamrihao K, Yang LL, Zhu WY, Zhang YG, Li L, Tang SK, Ningthoujam DS, Li WJ (2013) *Streptomyces hundungensis* sp. nov., a novel actinomycete with antifungal activity and plant growth promoting traits. *J Antibiot*, 66: 205-209

[6] Nimaichand S, Sanasam S, Zheng LQ, Zhu WY, Yang LL, Tang SK, Ningthoujam DS, Li WJ (2013) *Rhodococcus canchipurensis* sp. nov., an actinomycete isolated from a limestone deposit site. *Int J Syst Evol Microbiol*, 63: 114-118

[7] Nimaichand S, Zhu WY, Yang LL, Ming H, Nie GX, Tang SK, Ningthoujam DS, Li WJ (2012) *Streptomyces manipurensis* sp. nov., a novel actinomycete isolated from a limestone deposit site in Manipur, India *Antonie van Leeuwenhoek*, 102: 133-139

STUDY OF PROKARYOTIC DIVERSITY FROM EXPLOSIVES CONTAMINATED SITE BY CULTURE DEPENDENT AND METAGENOMIC APPROACH

P. Yash*, R. Mathan, P. Mual, S. Mayilraj, K. Murthi
Institute of Microbial Technology-Microbial Type Culture Collection, Sector 39A, Chandigarh160036, India
Email: Yashpal1591@imtech.res.in

Military explosives like RDX, HMX and PBX are nitro amine compounds which are recalcitrant toxic to environment and human health. Manufacturing sites of military explosive often leads to seepage of toxic nitro amines and nitro aromatic compounds into soil and ground water which ultimately reach human population. In this study an attempt is made to identify and culture organisms which are capable of degrading military explosives by using conventional culturing and metagenomics approaches to decipher the prokaryotic diversity of explosives manufacturing sites. Two explosive manufacturing sites were selected from India (Ordnance factory Nagpur and TBRL Panchkula) which manufactures RDX and HMX, various soil and water samples were collected from different parts of the manufacturing plant. For culture dependent study samples were plated on various media formulations along with broth consisting of varying concentrations of RDX and HMX to target the bacterial communities which specifically grow on explosive compounds. To culture uncultivable organisms which can withstand explosives, soil sample from the sites were directly sprinkled onto phyta gel plate consisting of RDX and HMX along with basal medium. Approximately 400 isolates were preserved from both sites. About 30 isolates from a total of 100 randomly selected for screening of RDX and HMX degradation showed positive results. The study also focuses on the culture independent prokaryotic diversity wherein soil and water samples were analyzed by metagenomic DNA sequencing and whole genome sequencing of three bacterial isolates WS2-TSB-28, WS2-TSB-13 & WS2-TSB-28 which utilize RDX and HMX. All the three samples were previously not reported to degrade or utilize RDX or HMX. In order to confirm the degradation potential of the various strains our work also targets the xplA gene cluster directly involved in the explosive degradation with the final aim of developing microbial consortia to mitigate the contamination of these chemicals.

NOVEL *SPHINGOBACTERIACEAE* RELATED TO *PEDOBACTER AGRI* ISOLATED FROM A FRESHWATER CREEK

E.A.Carson*, D.M.Snyder, J.D.Newman
Biology Department, Lycoming College, Williamsport, PA 17701, USA
Email: caremil@lycoming.edu; newman@lycoming.edu

As part of an undergraduate microbiology course, a pink pigmented, Gram-staining negative, rod-shaped, non-motile bacterial strain, designated R20-19 isolated from a creek in north-central Pennsylvania during the summer of 2011. Comparative 16S rRNA sequences identified the closest match to several *Pedobacter* species. Full genome sequencing of the isolate and reference type strains revealed an approximately 80% average amino acid identity (AAI) relative to other pink-pigmented species *Pedobacter agri*, *Pedobacter ginsenosidimutans* and *Pedobacter borealis*, well below the 95% threshold for different species. Genomic comparison to the yellow-pigmented type species *Pedobacter heparinus*, revealed an Average Amino Acid Identity (AAI) below 70%, which suggests that these organisms should be in a separate genus. Based on the genotypic and phenotypic results of this study, strain R20-19 represents a novel species within the *Sphingoobacteriaceae*. Many *Pedobactetr* species are highly divergent from the type species suggesting that significant reclassification into new genera is required.

REINSTATEMENT OF THE GENUS *KAISTELLA* AND PROPOSAL OF THE FAMILY, *CHRYSEOBACTERIACEAE*.

D.C.Frantz*, J.Clayton, J.E.Krebs, J.D.Newman
Biology Department, Lycoming College, Williamsport, PA 17701, USA
Email: fradevi@lycoming.edu; newman@lycoming.edu

The identification of a novel strain belonging to the *Flavobacteriaceae* led to comparative phenotypic and genomic studies with closely related reference strains that had recently undergone reclassification. The genus *Kaistella* was established in 2004 with the description of *K. koreensis* [1]. The genus *Sejongia* was established in 2005 with the description of *S. antarctica* and *S. jeonii* [2]. These species were moved into the genus *Chryseobacterium* in 2009 [3,4] based on their similarity to *C. haifense* which itself is only distantly related to *C. gleum*, the type species of the genus. Many other species that cluster with this group have also been assigned to the genus *Chryseobacterium*. We sequenced the genomes of six members of the cluster as well as eight other members of the genus *Chryseobacterium*. Here we show that this cluster forms a monophyletic group with characteristics such as small genome size, fatty acid composition, antibiotic sensitivity and a lack of flexirubin pigment production that distinguish the group from "true" *Chryseobacterium* species. We propose that the genus *Kaistella* be reinstated with *K. koreensis* as the type species, and that the former members of the genus *Sejongia*, as well as nine *Chryseobacterium* species, including *C. solincola, C. haifense*, and *C. palustre* should be moved into the genus *Kaistella*. The genomic distance from other members of *Flavobacteriaceae* also suggests that *Chryseobacterium*, *Kaistella* and related genera should be placed into a new family, proposed to be called the *Chryseobacteriaceae* fam. nov.

References

[1] Kim, M., Im, W., Shin, Y., Lim, J., Kim, S., Lee, B., Park, M., Lee, K. and Lee, S. (2004) *Kaistella koreensis* gen. nov., sp. nov., a novel member of the Chryseobacterium–*Bergeyella*–*Riemerella* branch. *Int. J. Syst. Evol. Microbiol* 54:2319-2324.

[2] Yi, H., Yoon, H.I. and Chun, J. (2005) *Sejongia antarctica* gen. nov., sp. nov. and *Sejongia jeonii* sp. nov., isolated from the Antarctic. *Int. J. Syst. Evol. Microbiol.* 55:409-416.

[3] Kämpfer, P., Lodders, N., Vaneechoutte, M. and Wauters, G. Transfer of *Sejongia antarctica*, *Sejongia jeonii* and *Sejongia marina* to the genus *Chryseobacterium* as *Chryseobacterium antarcticum* comb. nov., *Chryseobacterium jeonii* comb. nov. and *Chryseobacterium marinum* comb. nov. *Int. J. Syst. Evol. Microbiol.*, 2009, **59**, 2238-2240.

[4] Kämpfer, P., Vaneechoutte, M., Lodders, N., De Baere, T., Avesani, V., Janssens, M., Busse H.J. and Wauters, G. (2009). Description of *Chryseobacterium anthropi* sp. nov. to accommodate clinical isolates biochemically similar to *Kaistella koreensis* and *Chryseobacterium haifense*, proposal to reclassify *Kaistella koreensis* as *Chryseobacterium koreense* comb. nov. and emended description of the genus *Chryseobacterium*. *Int. J. Syst. Evol. Microbiol.* 59:2421-2428.

GENOMIC AND PHENOTYPIC CHARACTERIZATION OF *EPILITHONIMONAS DIEHLI* SP. NOV. FROM A FRESHWATER CREEK.

K.T.Jacobs* and J.D.Newman
Lycoming College, Williamsport PA 17701 USA
Email: jackyle@lycoming.edu; newman@lycoming.edu

A Gram-staining-negative, yellow-orange pigmented bacterial strain, designated FH1 was isolated from Fox Hollow in Williamsport, PA during an undergraduate microbiology course. The 16S rRNA sequence of strain FH1 was most similar to that of *Epilithonimonas lactis* (98.1%) and *Epilithonimonas ginsengisoli* (97.1%) in the family *Flavobacteriaceae*. The genomes of FH1 and *E. lactis* were sequenced, assembled, annotated and compared to each other and the type species of the genus, *E. tenax*. The estimated DNA-DNA hybridization value between FH1 and *E. lactis* was 33.1 as calculated by the DSMZ Genome-Genome Distance Calculator. The Average Nucleotide Identity (ANI) was 85.6. Both values are well below the threshold for separate species. Comparison of annotated gene sets determined that the 4.0 Mbp genome of FH1 contained 632 genes not found in either of the other *Epilithonimonas* sequenced genomes. Phenotypic comparisons identified a several distinguishing characteristics that support the hypothesis that isolate FH1 represents a novel species in the genus *Epilithonimonas* for which the name *Epilithonimonas diehli* sp. nov. is proposed.

TWO NOVEL FLAVOBACTERIACEAE RELATED TO *FLAVOBACTERIUM HYDATIS* AND FLAVOBACTERIUM HIBERNUM ISOLATED FROM A FRESHWATER CREEK

J.R.Miller*, A.T.Smith, J.D.Newman
Biology Department, Lycoming College, Williamsport, PA 17701, U.S.A
Email: miljaco@lycoming.edu; newman@lycoming.edu

As part of an undergraduate microbiology course, two yellow-orange pigmented, Gram-staining negative, rod-shaped, motile bacterial strains, designated JRM and KMS were isolated from a creek in north-central Pennsylvania during the winter of 2014. Growth was observed at temperatures 4-30 °C and an optimal pH of 6-9. Comparative 16srRNA sequences identified the closest match to *Flavobacterium hibernum* and *Flavobacterium hydatis*. Full genome sequencing of the two isolates and the reference type strains was completed and an estimated DNA-DNA Hybridization (eDDH) was calculated among them using the Genome-to-Genome- Distance Calculator. Strains JRM and KMS had an eDDH value of 74.6%, above the 70% for inclusion in the same species. When compared to JRM and KMS, *Flavobacterium hibernum* and *Flavobacterium hydatis* had GGDC values of under 70%. ANI was completed with the full genomes of JRM and KMS versus *Flavobacterium hibernum* and *Flavobacterium hydatis* with the results being under the threshold value for the same species. Genomic comparison to the type species *Flavobacterium aquatile* LMG 4008^{T}, revealed an Average Amino Acid Identity (AAI) below 70%, which suggests that this organism should be in a separate genus from *Flavobacterium aquatile*. Based on the genotypic and phenotypic results of this study, strains JRM and KMS represent a novel species within the *Flavobacteriaceae*. Many *Flavobacterium* species are highly divergent from the type species suggesting that significant reclassification into new genera is required.

NOVEL FLAVOBACTERIACEAE ISOLATED FROM A FRESHWATER CREEK

V.M.Wilson*, N.L.Marianelli, J.D.Newman
Biology Department, Lycoming College, Williamsport, PA 17701, U.S.A
Email: wilvict@lycoming.edu; newman@lycoming.edu

As part of an undergraduate microbiology course, a yellow-orange pigmented, Gram-staining negative, rod-shaped, motile bacterial strain, designated NLM, was isolated from a creek in north-central Pennsylvania during the winter of 2015. The 16S rRNA gene sequence was most similar to *Flavobacterium resisten*s BD-b365^{T} at 97.69% and was 94.77% similar to the type species *Flavobacterium aquatile* LMG 4008^{T}. Genomic comparison showed a 67.8% average amino acid identity (AAI), 77.8% average nucleotide identity (ANI), and 20.1% DNA-DNA hybridization (eDDH) to *Flavobacterium aquatile* LMG 4008^{T}. The low AAI value in particular suggests that this organism should be in a separate genus from *Flavobacterium aquatile*. Strain NLM was similar to many current *Flavobacterium* species but different from *Flavobacterium aquatile* in that it contained flexirubin-type pigments in addition to carotenoids, and lacked a rhodopsin homolog.

Growth was exhibited from 4-30°C (optimum of 20-30°C) and from a pH range of 5-10. Antibiotic resistance was observed for amoxicillin, clavulanic acid, kanamycin, and streptomycin. On Biolog GenIII plates, strain NLM showed significant utilization of the carbohydrates dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-melibiose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, α-D-glucose, D-mannose, D-fructose, and D-galactose. Based on the genotypic and phenotypic results of this study, strain NLM represents a novel species within the *Flavobacteriaceae*. Many *Flavobacterium* species are highly divergent from the type species suggesting that significant reclassification into new genera is required.

STUDY OF PROKARYOTIC DIVERSITY FROM THE MARINE HABITATS OF THE CENTRAL WEST COAST OF INDIA USING CULTURE DEPENDENT APPROACH

A. Verma*, A.K. Ojha, S.S. Sundharam, Y. Pal and S. Krishnamurthi
Microbial Type Culture Collection and Gene Bank, CSIR-Institute of Microbial Technology, Sector 39A, Chandigarh-160036
Email: ashish.verma02@imtech.res.in

With the advent of high throughput sequencing in the past decades, a number of candidate phyla have been discovered on the basis of 16S rRNA gene sequences. But in order to study their physiological and functional aspect, axenic cultures provide the undisputed backbone. With just ~12,000 validly described species of *Bacteria* and *Archaea*, a huge void is there to be filled with the discovery of novel taxa in the coming years. With the current estimated scenario of trillion species being present on Earth, it will take many centuries to harness the dark microbial matter. In the present work, an attempt has been done to culture some novel bacterial and archaeal taxa from different unexplored marine habitats of Central West coast of India using a combination of different marine environment mimicking culture media. A total of 717 isolates from soil (three mangroves and one salt pan) and epiphytic associated bacteria from the surface of macro-algal samples have been purified and preserved. Preliminary characterization of a total of 315 isolates (from five samples) were completed using a set of techniques such as partial 16S rRNA gene sequencing, MALDI-TOF and fatty acid profile analysis. At the phyla level in mangrove soils, *Firmicutes* (48-62%) were found to be dominated followed by *γ-Proteobacteria* (24-26%), *Actinobacteria* (6-20%), *α-Proteobacteria* (1-5%), *Flavobacteria* (5%) and *Actinomycetes* (1%), while the surface of macro-algal samples were found to be dominated by *γ-Proteobacteria* (35-59%) followed by *Actinobacteria* (20%), *Firmicutes* (9-43%), *α-Proteobacteria* (2-9%) and *β-Proteobacteria* (3%). At the genera level, a total of 28 and 21 genera were found in mangrove soils and macro-algal surface respectively. From the characterized isolates, a total of 15 strains were found to be novel both at the species and genus level. Some of these isolates are in the process of complete polyphasic characterization in addition to whole genome sequencing. Such a study of unexplored marine habitats can help us to better understand the key microbial populations involved in ecosystem functioning and might lead to discovery of important biomolecules.

EXPLOSIVE INCREASE IN THE DISCOVERY OF POTENTIAL NEW *ARCOBACTER* SPECIES.

M.J. Figueras*[1], A. Pérez-Cataluña[1], N. Salas-Massó[1], F.J. García[3], R. Alonso[4], A. Fernández[4] and J.M. Romalde[2].

[1] Facultad de Medicina y Ciencias de la Salud, IISPV, Universidad Rovira i Virgili (URV), Reus, Spain; [2] Facultad de Biología. Universidad de Santiago de Compostela (USC), Santiago de Compostela, Spain; [3]Laboratorio Central de Veterinaria de Algete, MAGRAMA, Madrid, Spain; [4]Facultad de Farmacia, Universidad del País Vasco (UPV/EHU), Vitoria, Spain.

Email: mariajose.figueras@urv.cat

The genus *Arcobacter* belongs to the family *Campylobacteraceae* and its species have been isolated worldwide from water, wastewater, marine ecosystems, food, animals and humans. Since the genus descriptions in 1991-92 with 4 species [1], the number remained constant up to 2005 when it increased to 6 species, but it raised exponentially with yearly new descriptions from 2009 (8 species) up to 2016 (23 species) [2-4]. Of the 23 species, 10 (43.5%) were discovered at the URV using the new molecular method we developed for species identification [4 and references therein]. In this work, we present the discovery of 15 additional potential new *Arcobacter* species isolated in 4 different laboratories in Spain. Eleven were found at the URV (2 isolated from wastewater and 9 from shellfish and water); two were isolated in EHU laboratory (one from wastewater and one from a carrot); another one was isolated at the USC laboratory from a scallop sample obtained from a hatchery in Norway, and the last one came from the MAGRAMA laboratory and was isolated from a southern elephant seal at the Avian island (Antarctic Sea). A phylogenetic tree constructed with five housekeeping genes show that all the strains form apparently new and distinctive taxa related to the species of the genus *Arcobacter*. The average nucleotide identity (ANI) and the *in silico* DNA-DNA hybridization (*is*DDH) are used to compare the genomes of the potential new species with the nearest known species. If all confirmed as belonging to this genus they will represent an explosive increase of 65%. The exploration of new culture approaches, molecular tools and habitats was the motor for this discovery.

References

[1] Vandamme, P., Vancanneyt, M., Pot, B., Mels, L., Hoste, B., et al. (1992) Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. *International Journal of Systematic Bacteriology* 42:344-56.

[2] Zhang, Z., Yu, C., Wang, X., Yu, S. and Zhang, X. H. (2015) *Arcobacter pacificus* sp. nov., isolated from seawater of the South Pacific Gyre. *International Journal of Systematic and Evolutionary Microbiology* 66:542–547.

[3] Whiteduck-Léveillée, K., Whiteduck-Léveillée, J-, Cloutier, M., Tambong, JT., Xu, R., et al. (2016) Identification, characterization and description of *Arcobacter faecis* sp. nov., isolated from a human waste septic tank. *International Journal of Systematic and Evolutionary Microbiology* 39:93-9.

[4] Levican, A., Rubio-Arcos, S., Martinez-Murcia, A., Collado, L. and Figueras, M. J. (2014). *Arcobacter ebronensis* sp. nov. and *Arcobacter aquimarinus* sp. nov., two new species isolated from marine environment. *Systematic and Applied Microbiol.* 38:30-35.

SCREENING OF CYPERMETHRIN DEGRADING GENE FROM *STREPTOMYCES ERYTHROGRISEUS* ADMT11 AND FROM SOIL METAGENOME

M. Tiwary* and A.K. Dubey
Netaji Subhas Institute of Technology, University of Delhi, Sector 3, Dwarka, New Delhi, India
Email: adubey.nsit@gmail.com

Pyrethroids represent 1.3 % of total global pesticide market and the major representative of this class is cypermethrin [1]. It's persistent and non-judicious use has created environmental and health problems. One of the upcoming approaches for remediation of the cypermethrin is microbial remediation. In environment, the cypermethrin is degraded initially into 3 PBA by soil microbes. This conversion requires the activity of esterase gene. The esterase gene responsible cypermethrin degradation can be screened and identified from culturable microbes and also directly from the soil metagenome. Isolation of such microbes, their screening for the degradation and then the gene screening, is time-intensive compared to if the gene interest is obtained directly from the metagenome. In the present study, cypermethrin-degrading strains of actinobacteria were isolated from the pesticide contaminated soil sample. One of the cypermethrin degrading actinobacterial isolate has been identified and screened for the gene involved in the degradation of cypermethrin. Similarly, the screening of esterase gene, responsible for cypermethrin degradation, has been done from metagenomic library of the soil sample contaminated with the pesticide. The presence of pesticides was found to cause significant affect on the microbial population in soil as determined through metagenomic approach, which involved sequencing of V3 and V4 region of 16S rRNA gene.

Reference

[1] Fenner, K., Canonica, S., Wackett, L.P., Elsner, M., (2013) Evaluating pesticide degradation in the environment: Blind spots and emerging opportunities. Science 341: 752-758.

CULTURING METHANE OXIDIZING BACTERIA (MOB) AMONGST THE UNCULTURED METHANOTROPHS FROM INDIAN WETLAND RICE FIELD SOIL

Pranitha Pandit and Monali Rahalkar*
MACS Agharkar Research Institute, G. G. Agarkar Road, Pune – 411004
Email: monalirahalkar@aripune.org

India and China are the largest rice producing nations, with major part of its agricultural land under rice cultivation but are least explored for methanotroph diversity and there has been no known report of mehanotroph cultivation from Indian rice fields to the best of our knowledge (3). Cultivation of methanotrophs has always been a challenging task and thus culture independent approaches have been well established for methanotroph diversity studies in the world (1,2). To explore the ability of MOB as methane mitigators we isolated and characterized the methanotrophs from different the rice rhizospheres of Western India. Successful isolation of 15 Type I, Type X and Type II methanotrophs belonging to the genera *Methylomonas, Methylocucumis* (putative novel genus), *Methylocaldum, Methylocystis* and *Methylosinus* have been carried out from about 20 wetland rice field samples. Among these genera *Methylomonas* and *Methylocystis* were found to be occurring commonly among all soil types collected from various paddy fields. The presence of these genera were confirmed by molecular methods like pmoA based clone library, T-RFLP and metagenomics for some of the soil samples that showed successful pmoA gene amplification. The methane oxidizing capacities of these bacteria were in the range of 10 to 50 fmoles CH_4/hr/cell. *Methylocucumis oryzae*, a putative novel genus novel species, was isolated from the rice rhizosphere soil from a western district of Maharashtra, India.

References

[1] Eller G, Kruger M, Frenzel P (2005) Comparing field and microcosm experiments: a case study on methano- and methylo-trophic bacteria in paddy soil. FEMS Microbiol Ecol 51:279–291
[2] Ma K, Conrad R, Lu Y (2013) Dry/wet cycles change the activity and population dynamics of methanotrophs in rice field soil. Appl Environ Microbiol 79:4932–4939
[3] Pandit PS, Rahalkar M, Dhakephalkar P, Ranade DR, Pore S, Arora P & Kapse N (2016) Deciphering community structure of methanotrophs dwelling in rice rhizospheres of an Indian rice field using cultivation and cultivation independent approaches. Microb Ecol 71: 634-644

STREPTACIDIPHILUS TORUNIENSIS SP. NOV., ISOLATED FROM A PINE FOREST SOIL

P. Golinska[1]*, M. Wypij[1*], H. Dahm[1] and Michael Goodfellow[2]
[1]*Department of Microbiology, Nicolaus Copernicus University, Torun, 87 100, Poland,*
[2] *School of Biology, Newcastle University, Newcastle upon Tyne, UK*
Email: golinska@umk.pl; mwypij@umk.pl

Acidophilic sporoactinobacteria which grow between pH 3.5 and 6.5 and optimally between pH 4.5 to 5.5 share key chemotaxonomic and morphological properties with streptomycetes (Kim et al. 2003). These organisms are numerous and widely distributed in acidic habitats, notably coniferous soils (Cho et al. 2008). Many of these acidophilic actinobacteria belong to the genus *Streptacidiphilus* which contains ten validly published species (Kim et al. 2003, Huang et al. 2004, Wang et al. 2006, Cho et al. 2008, Golinska et al. 2013a,b).

Two acidophilic sporoactinomycete strains, NF37^T and NA14, were isolated, respectively from fermentation litter and mineral layers of a pine forest soil in Poland and their taxonomic position established using polyphasic approach. Chemotaxonomic and morphological properties of the isolates were consistent with their classification in the genus *Streptacidiphilus*. 16S rRNA gene sequence analysis of the isolates showed that they had identical sequences and formed a branch within the evolutionary variation occupied by the genus *Streptacidiphilus*. The strains were most closely related to *Streptacidiphilus neutrinimicus* DSM 41755^T (99.4 and 99.9 % similarity, respectively). DNA:DNA relatedness data showed that isolate NF37^T and the type strain of *S. neutrinimicus* belonged to markedly distinct genomic species. In addition, the isolates were distinguished readily from their closest phylogenetic neighbours in the *Streptacidiphilus* gene tree using a phenotypic features. Based on the combination of chemotaxonomic, phenotypic and genotypic data isolates NF37^T and NA14 are considered to represent a novel species within the genus *Streptacidiphilus* for which the name *Streptacidiphilus torunensis* sp. nov. is proposed with isolate NF37^T as the type strain.

References

[1] Kim, S.B., Lonsdale, J., Seong, C-N., Goodfellow, M. (2003) *Streptacidiphilus* gen. nov., acidophilic actinomycetes with wall chemotype I and emendation of the family *Streptomycetaceae* (Waksman and Henrici 1943)^{AL} emend. Rainey et al. 1977. *Antonie van Leeuwenhoek* 83:107–116

[2] Cho, S-H., Han, J-H., Ko, H-Y., Kim, S.B. (2008) *Streptacidiphilus anmyonensis* sp. nov., *Streptacidiphilus rugosus* sp. nov. and *Streptacidiphilus melanogenes* sp. nov., acidophilic actinobacteria isolated from *Pinus* soils. *International Journal of Systematic Evolutionary Microbiology* 58:1566-1570

[3] Huang Y, Cui Q, Wang L, Rodriguez C, Quintana E, Goodfellow M, Liu Z (2004) *Streptacidiphilus jiangxiensis* sp. nov., a novel actinomycete isolated from acidic rhizosphere soil in China. Antonie van Leeuwenhoek 86:159–165

[4] Wang L, Huang Y, Liu Z, Goodfellow M, Rodriguez C (2006) *Streptacidiphilus oryzae* sp. nov., an actinomycete isolated from rice field soil in Thailand. *International Journal of Systematic Evolutionary Microbiology* 56:1257–1261

[5] Golinska P, Ahmed L, Wang D, Goodfellow M (2013a) *Streptacidiphilus durhamensis* sp. nov., isolated from a spruce forest soil. *Antonie Van Leeuwenhoek* 104:199–206

[6] Golinska P, Kim B-Y, Dahm H, Goodfellow M (2013b) *Streptacidiphilus hamsterleyensis* sp. nov., isolated from a spruce forest soil. *Antonie Van Leeuwenhoek* 104:965–972

GENOMIC ENCYCLOPEDIA OF BACTERIAL AND ARCHAEAL TYPE STRAINS, PHASE III: THE GENOMES OF SOIL AND PLANT-ASSOCIATED AND NEWLY DESCRIBED TYPE STRAINS

W.B. Whitman[1]*, T. Woyke[2], Y.-G. Zhou[3], H.-P. Klenk[4], N. Shapiro[2] and N.C. Kyrpides[2]
[1] Department of Microbiology, University of Georgia, Athens, GA; [2] DOE Joint Genome Institute, Walnut Creek, CA; [3] China General Microbiological Culture Collection Center (CGMCC), Institute of Microbiology, Chinese Academy of Sciences, Beijing, China; [4] School of Biology, Newcastle University, Newcastle upon Tyne, UK
Email: whitman@uga.edu

The Genomic Encyclopedia of *Bacteria* and *Archaea* (GEBA) project was launched by the JGI in 2007 as a pilot project to sequence about 250 bacterial and archaeal genomes of elevated phylogenetic diversity. Here, this approach was extended to type strains of prokaryotes associated with soil or plants and their close relatives as well as type strains from newly described species [1]. Individual investigators were invited to submit DNA from any of these type strains to JGI for sequencing and annotation. Since the project began in the Fall of 2013, individual investigators proposed 852 type strains for genome sequencing, and 588 of these strains were approved. The major reason the projects were not approved was that sequencing was in progress elsewhere. Sequences for 256 genomes have been completed or are in progress. Projects approved were largely for type strains from soils, plant associated and saline soils and were contributed by investigators from 14 nations, chiefly India, Spain, United Kingdom, South Africa, and China. In addition, approval was obtained for sequencing 328 type strains provided by the China General Microbiological Culture Collection Center (CGMCC), which possesses a large collection of type strains isolated in China. Sequences for 270 of these have been completed or are in progress. Therefore, this project has significantly increased the number of genome sequences for type strains, especially among plant and soil associated species.

References

[1] Whitman, W.B., Woyke, T., Klenk, H.-P., Zhou, Y-G., Lilburn, T.G., Beck, B.J., Kyrpides, N.C. (2015). Genomic Encyclopedia of Bacterial and Archaeal Type Strains, Phase III: the genomes of soil and plant-associated and newly described type strains. *Standards in Genomic Sciences*, *10*(1). doi:10.1186/s40793-015-0017-x

PROPOSAL FOR *"STREPTOMYCES PSEUDOACISDISCABIEI"* SP. NOV., AS A NOVEL PHYTOPATHOGENIC STREPTOMYCETE

A. Alejo-Viderique[1,2]*; L.A. Maldonado[3,4]; R. Riesco-Jarrín[2]; M.E. Trujillo[2]; E.T. Quintana[1]
[1]Laboratorio de Investigación en Actinobacterias, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico; [2]Departamento de Microbiología y Genética, Edificio Departamental, Lab. 214, Campus Miguel de Unamuno, Universidad de Salamanca, Salamanca, España; [3]Facultad de Química, Universidad Nacional Autónoma de México, Mexico City, Mexico. [4]Rectoría-Secretaría General, Universidad Autónoma Metropolitana, Mexico City, Mexico.
Email: erika_quintana@hotmail.com

Potato common scab (PCS) is an important economical worldwide disease [1]. PCS is caused by bacteria of the genus *Streptomyces* mainly by three species: *S. scabiei*, *S. acidiscabies* and *S. turgidiscabies* [2]. In this study, a polyphasic approach was conducted on four strains isolated from potatoes with visible common scab lesions that were collected in Ahome, Sinaloa, Mexico. Genotypic traits, namely REP-PCR and 16S rRNA gene sequence analysis of the four actinobacteria showed that they were closely related to the clade of *S. acidiscabies* according to Labeda and colleagues [3]. However, further studies to unravel the intrinsic relationships of the isolates and *S. acidiscabiei* based on a MLST analysis, the percentage of DNA:DNA hybridization and the value of the Average Nucleotide Index (ANI) from one of the four strains confirmed that the isolates should be assigned to a novel center of taxonomic variation within the *S. acidiscabiei* 16S rRNA gene clade. The complete polyphasic study not only identified the causal agent of PCS in Ahome Sinaloa but also fully supports the proposal of *Streptomyces pseudoacidiscabiei* as a new pathogenic species within the genus *Streptomyces*. Extended studies are required to establish the role of this putative novel pathogenic species as the main causative agent of PCS in the state of Sinaloa, Mexico.

References

[1] Wanner, L. A. (2010). A survey of genetic variation in *Streptomyces* isolates causing potato common scab in the United States. *Phytophatology*, **96**:1363-1371.
[2] Loria, R., Kers, J. and Joshi, M. (2006). Evolution of plant pathogenicity in *Streptomyces*. *Annual Reviwes in Phytopatholy*, **44**: 469-487.
[3] Labeda, D.P., Goodfellow M., Brown, R., Ward A.C., Lanoot B., Vanncanneyt M., Swings J., Kim S.B., Chun J., Tamura T., Oguchi A., Kikuchi T., Kikuchi H., Nishii T., Tsuji K., Yamaguchi Y., Tase A., Takahashi M., Sakane T., Suzuki I., Hatano K. (2011). Phylogenetic study of the species within the family *Streptomycetaceae*. *Antonie van Leeuwenhoek*, **101**, 73-104.

PARTICIPATION OF MEXICAN TOP-CLASS STUDENTS IN AN INTERNATIONAL SYSTEMATICS FORUM AS A WAY TO IMPROVE THEIR SCIENTIFIC AND TECHNOLOGICAL BACKGROUND ON THEIR ONGOING PROJECTS

A. Alejo-Viderique[1]; L. Contreras-Castro[1]; J. J. Luna-Castillo[1]; L. A. Medina-Romero[1]; A. F. Muñoz-Canedo[1]; G. Santiago-Cruz[1]; L.A. Maldonado[2,3]; E.T. Quintana[1]*

[1]Laboratorio de Investigación en Actinobacterias, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico; [2]Facultad de Química, Universidad Nacional Autónoma de México, Mexico City, Mexico; [3]Rectoría-Secretaría General, Universidad Autónoma Metropolitana, Mexico City, Mexico.

Email: equintanac@ipn.mx

Two premises lay behind this ongoing project (Grant C291045.86/2016 – SIP-2016-RE/046): (a) the lack of opportunities to promote microbial systematics locally and externally among students and (b) the lack of mobility for young talented students to attend international microbial systematics meetings/symposia. That in mind, this ongoing project targeted six-top class students from any level (ie. undergraduates, masters and PhD) to attend BISMiS-2016 to fulfill the "Systematic" needs of their projects either by interacting or promoting their ongoing lab works. Projects include issues related to the diversity, isolation, MLST, genome sequencing, or phylogeny of particular groups among the *Actinobacteria* [1]. Our group has extensively and almost solely concentrated on the study of the *Actinobacteria* for several years [2] and it is our view that attending BISMiS-2016 should provide the attendees with the perfect scenario to complement their dissertation projects from an academic-research training point of view and should also reinforce the urgent need to attend international microbial systematics meetings.

References

[1] Stackebrandt, E., Rainey, F. A., Ward-Rainey, N. L. (1997). Proposal for a New Hierarchic Classification System, *Actinobacteria* classis nov. *International Journal of Systematic Bacteriology*, **47**: 479-491.

[2] Maldonado, L. A., Quintana, E. T. (2014). Actinobateria in Mexico. *The Microbial Taxonomist Newsletter*, **7**: 3-4.

ON THE PHENOTYPIC TRAITS OF STRAINS ASSIGNED TO *SALINISPORA ARENICOLA* SPECIES

G. Santiago-Cruz[1]; A. F. Muñoz-Canedo[1]; M. E. Esteva-García[2]; L.A. Maldonado[3,4]*; E.T. Quintana[1]
[1]Laboratorio de Investigación en Actinobacterias y [2]Laboratorio de Genética Microbiana, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico City, Mexico; [3]Facultad de Química, Universidad Nacional Autónoma de México, Mexico City, Mexico. [4]Rectoría-Secretaría General, Universidad Autónoma Metropolitana, Mexico City, Mexico.
Email: luis_angel_maldonado@hotmail.com

Actinobacteria recovered from marine environments have received special attention in recent years due to their unique metabolic properties. The first actinobacteria genus to be marine-adapted, ie. *Salinispora* [1], has proven the latter point mostly because of the exclusive secondary metabolites that the three (at time of writing) species can produce. In the present study, 75 actinobacteria were recovered from subtropical sediment collected in Baja California Sur, Mexico and studied for their enzymatic and phenotypic properties while their relationships established using phylogenetic (genetic) approaches. Sixty-six strains were found to be related to the species *S. arenicola* and closely related to the "so-scalled" ecotype A. An evaluation of the enzymatic profile for the 66 strains for amylases, cellulases, lipases and proteases showed that 100% of the strains produced amylases and lipases; only 7.5% produced proteases and none produced cellulases. In addition, an IPS characterization study of all the isolates was also conducted and revealed an unexpected morphological plasticity for the 66 strains. Despite their chameleonic-plasticity morphological state, it is proposed that the ISP media 1, 5 and 7 could be useful to study the phenotypic features of *Salinispora*. Phylogenetic analysis based on 16S rRNA gene sequences, genome sequencing and morphological features should definitively be considered for the description of putative novel species and/or a reappraisal of the minimal standards on salinisporae delineation [2].

Reference

[1] Maldonado, L. A.; Fenical, W.; Jensen, P. R.; Kauffman, C. A.; Mincer, T. J.; Ward, A. C.; Bull, A. T. & Goodfellow, M. (2005). *Salinispora arenicola* gen. nov., sp. nov. and *Salinispora tropica* sp. nov., obligate marine actynomycetes belonging to the family *Micromonosporaceae*. *International Journal of Systematic and Evolutionary Microbiology*, **55**: 1759 – 1766.

[2] Goodfellow, M., Jensen, P. R., Maldonado, L. A. (2014). Genus XIV *Salinispora*. Bergey´s Manual of Systematic Bacteriology: Volume 5: The Actinobacteria. Springer Science & Bussines Media. pp. 1118-1122.

EVOLUTIONARY RELATIONSHIPS AMONG ROOT-NODULATING BACTERIA OF FENUGREEK (*TRIGONELLA FOENUM-GRAECUM*) CULTIVATED IN DIFFERENT PARTS OF INDIA

Praveen Rahi*, Krupa Parmar, Ashwini Hagir, Mitesh Khairnar and Yogesh S. Shouche
Microbial Culture Collection, National Centre for Cell Science, Pune-411021, India
Email: praveen@nccs.res.in

Fenugreek (*Trigonella foenum-graecum*) is an annual, self-pollinating legume plant. In order to determine the evolutionary relationships among the root-nodulating bacteria associated with fenugreek, a total of 143 isolate were obtained from fenugreek root nodules collected form 07 locations in Maharashtra, 02 Himachal Pradesh and 02 Haryana. The isolates were characterized by using MALDI-TOF MS. Comparison of MALDI-TOF MS spectra of isolates with Biotyper 3.1 database and in-house database of the Microbial Culture Collection indicated that most of the isolates belong to the genus *Ensifer* (118), and few isolate belong to *Rhizobium* (18), *Enterobacter* (03) and *Ochrobactrum* (01). Five isolates showed no reliable identity to the strains available in both databases. Representative isolates of all genus from each location were selected for phylogenetic analyses based on 16S rRNA, housekeeping genes (*atpD* and *recA*) and functional genes (*nodC* and *nifH*) sequencing. Functional genes *nodC* and *nifH* could be amplified for the isolates *Ensifer*, and not for the isolates of other genera, indicating that isolates belonging to *Ensifer* are the real symbionts of fenugreek, isolates of remaining genera might be present in the nodules as endophytes. The isolates of *Ensifer* were only considered for further analysis. Phylogenetic analyses based on the sequences of core-genome genes (16S rRNA, *atpD* and *recA*) exhibited the presence of three genotypes of fenugreek symbionts, placed with type strains of *E. meliloti* and *E. kummerowiae*. The analysis based on *nodC* and *nifH* genes placed the isolates under two clusters along with strains of *E. meliloti*, *E. kummerowiae* and *E. medicae*. Overall, the study indicated that fenugreek symbionts isolated from different parts of India are closely related to *E. meliloti*, comprising three different sub-lineages. A common *E. meliloti* sub-lineage in all locations of Maharashtra, which is diverse from that of Northern India indicated biogeographic pattern of distribution fenugreek symbionts.

CULTIVABLE MICROBIAL COMMUNITIES ASSOCIATED WITH CELLULAR PHONES

Praveen Rahi*, Rashmi Kurali, Mitesh Khairnar, Aabeejjeet N. Pansare and Yogesh S. Shouche
Microbial Culture Collection, National Centre for Cell Science, Pune-411021, India
Email: praveen@nccs.res.in

Cellular phones are the universally owned gadgets. The number of cellular phones is almost same as total human population of the world, which is expected to increase 1.5 mobile devices per capita by 2020 (Cisco Systems Inc., 2016). The study reports the diversity of microbes associated with cellular phones using cultivation based methods. Cellular phones of 27 individuals in Pune, India, were sampled and a total of 515 isolates of bacteria and 28 fungi were obtained representing different morphotypes. Whole cells MALDI-TOF MS profiling of the bacterial isolates could identify 355 isolates (69 %), 209 species level (41 %) and remaining 146 only to genus level. The isolates belong to 20 different genera. *Staphylococcus*, *Micrococcus*, *Bacillus*, *Pseudomonas* and *Kocuria* were the major genera among bacteria and *Candida* among fungi. A PCA dendrogram was generated based on their MALDI-TOF MS spectra of isolates not identified by MALDI-TOF MS. The isolates were placed into 32 different clusters. The representative strains of each group were selected for 16S rRNA gene sequencing for identification. Two isolates S5H2222 and S2T63 showed less than 98.5 % sequence similarity to closest type strains of validly published species indicating that they could be potential new species. Based on the phenotypic and chemotaxonomic properties, strain S5H2222 and S2T63 could be distinguished from the recognized species of the genus *Lysinibacillus* and genus *Microbacterium* respectively. The name *Lysinibacillus telephonicus* sp. nov. is proposed for strain S5H2222 and *Microbacterium telephonicus* sp. nov. is proposed for strain S2T63. A new fungal species *Pyrenochaeta telephoni* was also described from the same study [1].

References

[1] Crous et al. (2015) Fungal Planet description sheets: 371-399. *Persoonia* 35: 264-327.

SPECIES LEVEL CLASSIFICATION OF '*CANDIDATUS*' BACTERIA: IS ACCESSING ONLY 16S RRNA GENE SEQUENCE DIVERSITY SUFFICIENT?

Amit Yadav*
Microbial Culture Collection, National Centre for Cell Science, Sai Trinity Complex, Pashan-Sus Road, Pashan, Pune 411 021 India
Email: amityadav@nccs.res.in

The 16S rRNA gene sequence is a preferred choice of microbial taxonomist to study bacterial taxa to begin with. The 16S rRNA gene sequence analysis enables one to identify rare bacterial genera and detect not-yet-cultivated taxa from environmental samples. Additional data collected from polyphasic approach help delineating the bacterial strain(s) to its deeper taxonomic status [1]. In case of '*Candidatus* Phytoplasma', a plant pathogenic, not-yet-cultivated bacterium; a new species description must contain 16S rRNA gene sequence that is < 97.5 % similar to any previously described phytoplasma species [2]. Further, the 16S rRNA RFLP scheme helps technically delineate phytoplasma strains to 'group' and 'sub-group' level [3]. Till now, more than 30 groups and 100 'sub-group' level phytoplasma strains have been described, which solely relied on 16S rRNA gene diversity and did not take into account of other biological characteristics. The sequence variation in 16S rRNA gene, due to its conserved nature, does not always provide the resolution to classify the phytoplasma strain to species or strain level. This may lead assigning or failing particular strain to its desired taxonomic designation, which should have been based on its biological characteristics in addition to 16S rRNA gene sequence data [4]. The guidelines on phytoplasma taxonomy should now incorporate the additional sequence data from phytoplasma gene(s) or genome sequences [4, 5]. We are attempting the classification of sugarcane phytoplasma based on secA, secY and Tu elongation factor genes in comparison with Cynodon white leaf (*Ca.* P. cynodontis) phytoplasma strains.

References

[1] Clarridge, J. E. III (2004). *Clinical Microbiology Review*, **17(4):** 840–862
[2] IRPCM Phytoplasma/Spiroplasma Working Team- Phytoplasma Taxonomy Group (2004). *International Journal of Systematic and Evolutionary Microbiology*, **54:** 1243-1255.
[3] Zhao, Y., Wei, W., Lee, I.-M., Shao, J., Suo, X. and Davis, R. (2009). *International Journal of Systematic and Evolutionary Microbiology*, **59:** 2582-2593
[4] Firrao, G., Martini, M., Ermacora, P., Loi, N., Torelli, E., Foissac, X., Carle, P., Kirkpatrick, B., Liefting, L., Schneider B., Marzachìg, C. and Palmano, S. (2013). *Systematic and Applied Microbiology*, **36(8):**539-48.
[5] Duduk, B. and Bertaccini, A. (2011). Phytopathogenic Mollicutes, **1(1):** 3-13

BISMiS-2016 National Student Travel Award Recipient

MECHANISMIC SURVIVAL OF A PGPR STRAIN *PSEUDOMONAS SIMIAE* AU ISOLATED FROM SEMI-ARID REGION OF RAJASTHAN, INDIA IN RESPONSE TO SALINITY STRESS

Anukool Vaishnav[1,2]* and Devendra Kumar Choudhary[2]
[1]ICAR-NBAIM, Maunath Bhanjan, Uttar Pradesh, 275103 India; [2]Amity Institute of Microbial Technology (AIMT), Block 'E-3', 4th Floor, Amity University Campus, Sector-125, Noida-201303, Gautam Buddha Nagar, UP, India
Email: ANUKOOLV7@gmail.com

Semi-arid regions are well understood for harsh variations in environmental conditions such as osmotic stress, temperature, nutrient limitations. Some plant growth promoting rhizobacteria (PGPR) possess potential to tolerate such climatic conditions. This study was focused on identification and characterization of salt tolerant bacteria from soybean rhizosphere in Bundi district, Rajasthan, India. A total of 43 bacterial isolates were recovered, out of which 19, 10, 8, 4, 2 isolates were found to tolerate 4, 6, 8, 10 & 12% NaCl respectively. Out of them, one isolate AU was able to retain PGP activities (IAA, Pi-solubilization and production of siderophore, ACC-deaminase & beneficial VOCs) up to 10% NaCl. Based on 16S rRNA sequencing, AU strain was most closely related to *Pseudomonas simiae* OLi type strain (AJ936933) with 99.93% similarity. In addition, various PGP genes (ACC-deaminase, tryptophan-2-monooxygenase, glucose-6 phosphate dehydrogenase, siderophore, alpha-amylase, nitrite reductase and proline) of AU isolate showed highest similarity with *P. simiae* WCS417 (CP007637) through sequencing of each gene. As a cellular response to salinity stress, AU isolate produced TonB and outer membrane receptor for iron transport, N-acetylmuramoyl-L-alanine amidase fusion and colanic biosynthesis UDP-glucose lipid carrier transferase like proteins identified using MALDI-TOF MS and increased the production of soluble sugars, free amino acids and proline contents. This is the first report on the study of the molecular and physiological mechanism adapted by *P. simiae* AU when exposed to contrasting salinities in external environment.

CONSORTIAL EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA FOR THE MANAGEMENT OF EARLY BLIGHT OF TOMATO INCITED BY *ALTERNARIA SOLANI*

Suleiman Abdullahi Suleiman*
Federal University Dutse, P.M.B. 7156, Jigawa State, Nigeria.
Email: suleiman.ab@fud.edu.ng

Rhizosphere bacteria are one of the most potential biological control agents in the plant disease disease protection. *Bacillus* and *Pseudomonas* species offer several advantages over other bacteria for protection against pathogens because of their ability to form endospores, and because of the broad-spectrum activity of their antibiotics [1]. They are reported to be effective in controlling soil-borne pathogens in the field. Based on the preliminary research proven, they could reduce 70% of the total soil-borne diseases and increase crop production up to 40% [2]. In this study, five soil samples from tomato rhizosphere were collected from Allahabad area of Uttar Pradesh State, India. Ten local bacteria were screened for their antagonism. Five promising antagonists exhibiting higher zone of inhibition (ZOI) (38mm and above) and percent disease control (ranging from 38.16 to 43.79%) were selected. These strains of *Bacillus subtilis* (Bs12 and Bs16) and *Pseudomonas fluorescens* (Pf2, Pf9 and Pf17) were tested individually and in combination for their effectiveness against early blight of tomato incited by *Alternaria solani* under *in vitro* and pot culture conditions. The results revealed that the strains of *Bacillus subtilis* and *Pseudomonas fluorescens* were compatible. Under *in vitro* conditions the combined application of Bs12+ Pf2 was found to effectively inhibit the mycelial growth of the pathogen and promote the growth of tomato seedlings when compared to application of individual strains of the antagonists. Further, a significant reduction in early blight incidence of tomato under greenhouse conditions was observed due to the combined application of Bs12+ Pf2. These findings suggest that synergistic consortia of biocontrol agents may be successfully employed as an eco-friendly strategy for the management of early blight of tomato.

References

[1] Naim, M., Ibrahim, A., Algam, A. and Abdallah, A. (2014) *In Vitro* Screening of *Bacillus* Isolates for Biological Control of Early Blight Disease of Tomato in Shambat Soil. Khartoum, Sudan.

[2] Hanudin, Marwoto,B., Hersanti, Muharam, A. (2012) Compatibity of *Bacillus subtilis* and *Pseudomonas fluorescens* in the management of wilt disease of potato caused by *Ralstonia solanacearum.* Indonesian Vegetable Research Institute. Sumedang, Indonesia.

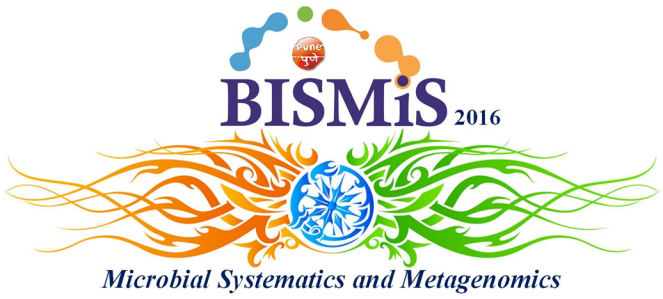
BISMiS-2016 Participants

BISMiS-2016 Participants

Name	Country	Email
Abhay Bajaj	India	abhaybajaj@gmail.com
Aharon Oren	Israel	aharon.oren@mail.huji.ac.il
Amaiza Munoz	Mexico	amaii_1973@hotmail.com
Amanda Alejo	Mexico	alejo_viderique_amanda@yahoo.com.mx
Amaraja Joshi	India	amarajaj@gmail.com
Amit Yadav	India	amityadav@nccs.res.in
Aniket Saraf	India	aniket3saraf@gmail.com
Anukool Vaishnav	India	anukoolv7@gmail.com
Ashish Verma	India	ashish.csirihbt@gmail.com
Bhagwan Rekadwad	India	rekadwad@gmail.com
Boris Vinatzer	USA	vinatzer@vt.edu
Brian Austin	UK	baustin5851@gmail.com
Brian Hedlund	USA	brian.hedlund@unlv.edu
Chintalapati Sasikala	India	sasikala.ch@gmail.com
David R Arahal	Spain	arahal@uv.es
Devin Frantz	USA	fradevi@lycoming.edu
Dhiraj Dhotre	India	dheerajdhotre@gmail.com
Dilip Ranade	India	drranade@gmail.com
Diptaraj Chaudhari	India	diptarajchaudhari89@gmail.com
Edward Moore	Sweden	erbmoore@ccug.se
Emily Carson	USA	eacarson429@yahoo.com
Erika Quintana	Mexico	erika_quintana@hotmail.com
Fred Rainey	USA	farainey@gmail.com
Hanna Dahm	Poland	dahm@umk.pl
Hans Peter Klenk	UK	hans-peter.klenk@ncl.ac.uk
Hitarth Bhatt	India	hitarth_bhatt@yahoo.co.in
Iain Sutcliffe	UK	iain.sutcliffe@unn.ac.uk
Jacob Miller	USA	miljaco@lycoming.edu

Name	Country	Email
Jayant Khire	India	jm.khire@ncl.res.in
Jeffrey Newman	USA	newman@lycoming.edu
Joerg Overmann	Germany	ans11@dsmz.de
Johana Luna	Mexico	johanita_lc15@hotmail.com
Jongsik Chun	Korea	jchun@snu.ac.kr
Kamlesh Jangid	India	jangidk@nccs.res.in
Kumari Richa	Italy	kumariricha.szn@gmail.com
Kunal Jani	India	kunaljaani@gmail.com
Kyle Jacobs	USA	jackyle@lycoming.edu
Lan Liu	China	liulan5@mail2.sysu.edu.cn
Laura Amrica Medina Romero	Mexico	laurakannon0@gmail.com
Luis Contreras Castro	Mexico	lucoca2000@hotmail.com
Magdalena Wypij	Poland	mwypij@umk.pl
Mahesh Chavadar	India	maheshchavadar@nccs.res.in
Maria Jose Figueras	Spain	mariajose.figueras@urv.cat
Marike Palmer	South Africa	marike.duplessis@fabi.up.ac.za
Martha Trujillo	Spain	mett@usal.es
Meenakshi Tiwary	India	tiwary.meenakshi@gmail.com
Monali Rahalkar	India	monarahul2002@yahoo.com
Neetha Joseph	India	neetha@nccs.res.in
Nimaichand Salam	China	s.nimaichand@hotmail.com
Nirjara Singhvi	India	nirjara20@gmail.com
Nisha Patel	USA	npatel@ou.edu
Om Prakash	India	prakas1974@gmail.com
Patricia Benito Santano	Spain	patriciabs@usal.es
Patrycja Golinska	Poland	golinska@umk.pl
Paul Lawson	USA	paul.lawson@ou.edu
Pelin Yilmaz	Germany	pyilmaz@mpi-bremen.de
Peter Kaempfer	Germany	peter.kaempfer@umwelt.uni-giessen.de

Name	Country	Email
Pierreedouard Fournier	France	pierre-edouard.fournier@univ-amu.fr
Piyush Raut	India	pwraut89@gmail.com
Prafulla Shede	India	prafullashede@gmail.com
Prashant Patil	India	prashantpatil@imtech.res.in
Prashant Singh	India	sps.bhu@gmail.com
Praveen Rahi	India	praveen@nccs.res.in
Rahul Sharma	India	rahulpremasharma@gmail.com
Raul Riesco Jarrin	Spain	shot89_1000@usal.es
Rohit Sharma	India	rohit@nccs.res.in
Rup Lal	India	ruplal@gmail.com
Shekhar Mande	India	shekhar@nccs.res.in
Shreyas Kumbhare	India	shreyas.kumbhare@gmail.com
Shrikant Pawar	India	pawar.shree@nccs.res.in
Snehal Kulkarni	India	snehal.pradhan@gmail.com
Srinivasan Krishnamurthi	India	kmurthi@imtech.res.in
Stefano Ventura	Italy	stefano.ventura@ise.cnr.it
Stephanus Venter	South Africa	fanus.venter@up.ac.za
Stephen Rudd	USA	srudd@pacificbiosciences.com
Svetlana Dedysh	Russian Federation	dedysh@mail.ru
Syed Dastager	India	sg.dastager@ncl.res.in
Tapan Chakrabarti	India	tapan@nccs.res.in
Vasudev Kshirsagar	India	vgkshirsagar@rediffmail.com
Victoria Wilson	USA	wilvict@lycoming.edu
Vipin Gupta	India	guptavipin142@gmail.com
Wenjun Li	China	liwenjun3@mail.sysu.edu.cn
William Whitman	USA	whitman@uga.edu
Yash Pal	India	yashpal1591@imtech.res.in
Yihuan Wang	China	wangyih6@mail2.sysu.edu.cn
Yogesh Shouche	India	yogesh@nccs.res.in



साई ट्रिनिटी कॉम्प्लेक्स, सुस रोड, पाषाण, पुणे- ४११ ०२१
Sai Trinity Complex, Sus Road, Pashan, Pune- 411 021
दुरभाष/ Tel: +91 20 25329000 | फ़ैक्स/ Fax: +91 20 25329001
ई-मेल/ e-mail: mcc@nccs.res.in | वेब/Web: www.nccs.res.in/mcc
एनसीसीएस भारत सरकार के जैवप्रौद्योगिकी विभाग का स्वायत्त संस्थान है.
NCCS is an Autonomous Institute of Department of
Biotechnology, Govt. of India