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MEETING ABSTRACTS

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Abstracts

Opening Keynotes

THE GENOMIC ENCYCLOPEDIA OF *BACTERIA* AND *ARCHAEA* PROJECT AND ITS USE FOR MICROBIAL TAXONOMY

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The Genomic Encyclopedia of *Bacteria* and *Archaea* (GEBA) started in 2007 with a pilot project for 165 type strain genomes to be sequenced and analyzed in a collaboration between the DOE Joint Genome Institute (JGI, Walnut Creek, CA) and the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig). Analysis of the first set of genome sequences confirmed the key idea of a significantly accelerated access to genomic diversity by phylogeny-driven selection of the sequencing targets, as well as rapid progress in the discovery of novel protein families [1]. Furthermore, the genome sequences were successfully used to support taxonomic revisions in problematic regions of the bacterial diversity [2,3], not least because of statistically well supported whole genome sequence-based phylogenies in combination with classical polyphasic taxonomic data. Inspired by the success of the pilot project, the GEBA project was meanwhile four times extended [4] to a total of 3250 type strain genomes, representing about 30% of all species with validly published names. The steadily increasing coverage of microbial diversity with complete and draft genome sequences in combination with improved genome sequence–based methods for species delimitation [5] and inference of reliable phylogenies [3] will contribute to qualitative progress in the description of novel species within the yet unexplored microbial majority.

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THE STATE OF MICROBIAL TAXONOMY TODAY

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Taxonomy provides a key role in the grand vision of modern microbiology that extends greatly beyond the simple naming of individual species or higher taxa. While naming items in the biological world is certainly important, taxonomy also provides an opportunity to organize our knowledge of prokaryotes in an informative manner. While in principle either artificial or natural classifications can be informative, experience has demonstrated that natural classifications based upon phylogeny are much more successful. The importance of phylogeny in taxonomy may be illustrated with the library metaphor. If we consider each bacterial species on earth a book in a library and our goal is to learn the entire contents of the library, there are two general strategies. In the first, we read each book. In the second, we assume that the books are arranged in the library according to some general rules or natural patterns. Thus, books with similar content might be arranged together on the same or neighboring shelves. In this case, it is only necessary to read parts of each book to infer the entire contents of the library. In this strategy, our goal can be achieved if we first discover the pattern and then read only those parts of each book likely to be unique. Phylogeny provides the pattern or general organizing principles for our library. Because of its availability, the 16S rRNA gene is the dominant phylogenetic marker in wide use. However, with the availability of genomic sequencing, we can look forward to much more robust trees with a much higher levels of resolution.

An ideal classification would use unambiguous and objective criteria that can be applied uniformly across all groups. Such a classification would have obvious advantages. It would be easy to use. Placement of new taxa would be simple. Expertise in classification of any particular lineage would be readily transferred to other lineages. The value of this approach is illustrated by the success of the Wayne et al. [1] proposal of DNA:DNA hybridization criteria for delineation of species. This proposal has been widely adopted, especially for the justification of creation of new species. However, the availability of genomes will allow us to examine the fundamental meaning of this classification with a rigor not possible before. The original proposal included two very different criteria for evaluating DNA hybridization. One examined the melting temperature of the hybrids, which reflects sequence similarity of the shared genes. This criterion most closely corresponds to relatedness of ancestry. The second evaluated the fraction of DNA that hybridized, which reflects similarity in gene content. While the high sequence similarity generally correlates with similar gene content, this relationship has not been critically examined for a variety of different lineages. Thus, while the criteria are clear, their interpretation remains ambiguous.

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Abstracts

Session 1 – Use of genomic sequences in microbial taxonomy

GENE-BY-GENE APPROACHES TO CHARACTERISING GENOMIC VARIATION IN BACTERIAL POPULATIONS

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The maturing of very high-throughput parallel sequencing technologies means that whole genome sequence (WGS) data are now available for very large numbers of isolates [1]. Although these data are mostly assembled from short-read sequences, current technology rapidly and inexpensively generates data corresponding to the great majority (>99%) of a bacterial genome to very high accuracy [2]. The challenge and opportunities now facing systematic microbiology are therefore intellectual rather than technical. Specifically, while WGS data are a mine of information, much of the data and analysis tools are inaccessible to many microbiologists, or accessible with only limited phenotype and provenance information. Bacterial genomes and, even more so, large collections of bacterial genomes need to be assembled, curated and disseminated to the wider community. Community users can then participate in, and contribute to, data analysis and curation [3]. It is also the case that most microbiological applications a limited amount of the information in the genome, perhaps only one or a few genes. To meet these needs we have developed a gene-by-gene 'population annotation' approach to WGS data [4] within our web-accessible open source platform, the Bacterial Isolate Genome Database (BIGSdb) on the PubMLST.org website [3]. This links sequence data of any type to provenance and phenotype data. The use of catalogues of sequence variation for each definable locus within a given subset of bacteria, from strains through to the whole domain, permits flexible and hierarchical genome analysis, including ribosomal MLST (rMLST) [5] and core-genome MLST (cgMLST) [4] enabling accurate isolate characterisation from 'domain to strain'.

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AVERAGE NUCLEOTIDE IDENTITY CALCULATIONS WITH TAXONOMIC PURPOSES: CASE STUDIES AT CECT

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Average Nucleotide Identity (ANI) was proposed almost ten years ago as a mean to compare genetic relatedness among prokaryotic strains [1]. It was found that values around 94% corresponded to the traditional 70% DNA-DNA hybridization (DDH) standard for prokaryotic species circumscriptions [1,2]. Keeping in mind that ANI calculations are only one of the many aspects and approaches that can be implemented from comparative genomics into taxonomy, an overview is given about its current usage at CECT, together with methodological indications from our own experience.

Our research group has implemented this methodology in several taxonomic studies that would have otherwise been addressed though wet DNA-DNA hybridization. Among the reasons for adopting this strategy was the publication of the paper describing the software JSpecies [3] and proposing that lower genome coverage could also yield reliable ANI values and genome coherence inference. Other good reasons were the reduction in price of genome sequencing costs and the hope that generated data could serve for other types of analysis and knowledge of the organisms sequenced.

Since June 2011 we have targeted eight bacterial groups totaling 23 strains of which 17 were new isolates and 6 type strains from reference species. Whole genome sequence data were obtained using two sequencing platforms. Sequence read archives are publicly available and cited in a total of six research articles. This communication gives insights about practical details concerning coverage, utility, costs, other outputs, etc. and demonstrates that it can be considered a routine approach in polyphasic taxonomy for prokaryotes, not requiring large investments in equipment or capacity building.

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GENOME SEQUENCE-BASED TAXONOMY: I WILL KNOW IT WHEN I SEE IT

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All taxonomies result from analysis of observational data. As John Locke posited [1], people make species, nature does not and people make boundaries of species and nature does not. Thus, uncertainty and subjectivity affect the process of establishing species [2] and determining strain identity [3], [4], [5], [6]. So too, the classification process with whole genome data is subjective. Metrics used in developing classifications are not uniform. The resulting topologies differ at least in detail. Boundaries delineating species are artificial constructs especially sensitive to classification method and number of sequences classified. Discrete boundaries are a human desire, not a natural phenomenon [7]. Use of whole genomes does not enable one to escape this subjectivity because there are no true objective means for doing comparisons. As with the conventions for assigning species names, the community could agree on a convention for "approved methodology". Whole genome sequencing alone is not the answer to the need for a mechanism for defining species in microorganisms. Why expect that a discrete species boundary exists in nature? The microbiologist, as always, must build, adapt, or choose a taxonomy to fit the need. Thus, too, for genome sequence-based taxonomy: I will know it when I see it.

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Taxono-genomics: an example of genomic data incorporation in bacterial taxonomy equation

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Thanks to the development of high throughput sequencers, whole bacterial genome sequencing (WGS), once a dream, has become a reality, with many derived applications in microbiology. With more than 12,000 genomes currently available, including those from most major human pathogens, it is time for WGS, which provides the ultimate genetic information of a strain in a reproducible way, to be considered as a potential taxonomic tool. Recently, by diversifying bacterial culture conditions we isolated more than 100 previously uncultivated bacteria. To classify the putative novel species that we cultivated, we used a polyphasic strategy that included phenotypic as well as genomic criteria, which we named taxono-genomics. Our approach was to compare genomes not only at the sequence similarity level, using the AGIOS parameter, but also in terms of main genome characteristics. However, as several other strategies and tools have been developed, a synthesis of the pros and cons of the various approaches is necessary and an agreement on the methods and parameters to be used should be found.

WHOLE GENOME ANALYSES SUPPORT THE TAXONOMIC SEPARATION OF "*RHODOCOCCUS EQUI*" FROM OTHER RHODOCOCCI

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Morphological and biochemical properties played a key role in prokaryotic systematics until the introduction of 16S rRNA gene sequencing in the 1980s [1]. *More recently, next generation sequencing (NGS) has been used to catalogue prokaryotic diversity from various sources and can be applied to prokaryotic systematics because* the genome sequences index entire micro-variation that can help identify the most reliable boundaries between different taxa. We applied whole genome sequencing to help resolve the taxonomic status of *Rhodococcus equi,* a Gram-positive mycolic acid containing actinomycete. We have sequenced the type strain of *R. equi*, $C7^T$ and performed phylogenetic analysis including other *R. equi* isolates, other genome sequenced rhodococci and closely related species using 400 conserved proteins. *R. equi* strains were grouped closely together but distinctly from other rhodococci and other related species, strongly supporting the recent proposal to reclassify it into a separate genus as "*Prescottella equi*" by Jones, *et al.* (2013)[2,3]. The name *Prescottella* has yet to be validated as the taxonomic status of *R. equi* awaits clarification [4].

R. equi is primarily a foal pathogen but has recently emerged as an important opportunistic human pathogen. The comparison of the *R. equi* genomes revealed a very high conservation of nucleotide and protein sequences within the species and only a few genes could be linked to the host jump from equine to human host. The diminishing cost of NGS and availability of advanced bioinformatic tools make it suitable for prokaryotic systematists to apply NGS for reliably defining new taxa and can also help identify the signature of host jumps (with geographic and temporal associations for some species) which can be exploited as potential diagnostic or taxonomic markers.

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WHOLE GENOME SEQUENCE ANALYSIS OF *KOSAKONIA RADICINCITANS*, A BACTERIAL STRAIN ASSOCIATED WITH BACTERIAL WILT DISEASED BANANA PLANT

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Bacterial wilt disease is considered as one of the most important bacterial diseases with a worldwide distribution. In this study, *K. radicincitans* was one of the bacterial species isolated from the endosphere of the bacterial wilt diseased banana plants. Herein, we report the annotated draft genome of UMEnt01/12 strain that may shed light on its role as a bacterial wilt-associated bacterium. To our knowledge, this is the first genome sequence analysis of a banana-associated bacterium from the genus *Kosakonia*. The genome comprises of 5 783 769 base-pair (bp) circular chromosome with 53.9 % GC-rich region. The UMEnt01/12 chromosome is made up of 5463 proteins-, 75 tRNAs- and 9 rRNAs- encoding sequence, giving a 89.0 % similarity to the UMEnt01/12 genome sequence. These proteins encode functions related to chemotaxis, adhesion, colonization, and biofilm formation. There are also proteins putatively involved in metabolisms such as utilization of carbohydrates in plant, oxidative stress, uptake of plant nutrients, nitrogen fixation process and type VI secretion system (T6SS). Based on the phenotypic characteristics and genome analysis of *K. radicincitans* UMEnt01/12 strain, it is suggested that this bacterial species has good adaptation and colonization properties to overcome environmental stresses and to compete with other microorganisms inhabiting the same niche in the host plant.

Microbial genomic taxonomy

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Genomic taxonomy is based on whole genome sequences. The determination of taxonomic similarity by means of several *in silico* tools [e.g. Average Aminoacid Identity (AAI), genome-to-genome distance (GGD), and supertrees] allows the identification and classification of prokaryotes [1]. However, the traditional phenotypic (and chemotaxonomic) characterization of prokaryotes has been expensive, time-consuming and restricted in scope to a limited number of features. In addition, most of the commercial systems applied for phenotypic characterization cannot characterize the broad spectrum of environmental strains. A reliable and possible alternative is to obtain phenotypic information directly from whole genome sequences. For instance, the analysis of 26 vibrio genomes revealed that all genes coding for the specific proteins involved in the metabolic pathways responsible for positive phenotypes of the 14 diagnostic features (Voges-Proskauer reaction, indole production, arginine dihydrolase, ornithine decarboxylase, utilization of myo-inositol, sucrose and l-leucine, and fermentation of d-mannitol, d-sorbitol, l-arabinose, trehalose, cellobiose, d-mannose and d-galactose) were found in the majority of the vibrios genomes. Vibrio species that were negative for a given phenotype revealed the absence of all or several genes involved in the respective biochemical pathways, indicating the utility of this approach to characterize the phenotypes of vibrios. Whole genome sequences represent an important source for the phenotypic identification of prokaryotes [2].

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PHYLO- AND COMPARATIVE GENOMICS OF THE *PANTOEA* CORE GENOME

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The delineation of bacterial genera with a limited set of characteristics can result in the artificial grouping of species. With the increased availability of whole genome sequences, various comparative and phylogenetic approaches to investigate the relatedness among bacterial strains have been suggested and used to define bacterial species [1]. It is believed that similar comparisons could be used to investigate the coherence amongst species within a genus. An essential first step to such comparisons involves determination of the core genome (i.e., genes that are present amongst all species in the genus). These genes are targeted as they collectively may represent the dominant evolutionary signal that depicts vertical descent [2]. In this study, the core genome of the genus *Pantoea* was determined and characterized in terms of its general cellular processes and functions using Clusters of Orthologous Genes (COG) associations. Our results indicated that the *Pantoea* core genome consisted of approximately 2500 genes. Of these the highest proportion belonged to the functional category of metabolism, followed by cellular processes and signalling, information storage and processing, and lastly by genes that are poorly characterized or uncharacterized. Comparisons of the *Pantoea* core genome with that of nine other bacterial genera revealed similar proportions of genes in the various functional catogories. The only exceptions were genera that have smaller core genomes, which tended to have a higher proportion of genes involved in information storage and processing. Phylogenetic analyses indicated that the evolutionary histories inferred for the various functional sets of genes were similar, although slight changes in sister relationships were observed for some of the species. Overall, our results significantly improved current knowledge regarding the function and evolution of the genomic component shared by all members of the genus, the implemention of which will be invaluable for defining genus boundaries in *Pantoea* and bacteria in general.

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MOLECULAR-GENETIC MARKERS IN DETERMINATION OF TAXONOMIC POSITION CYANOBACTERIA FROM SUBSECTION III

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The classification of *Cyanobacteria* is currently intricate, not only in connection with the problem of species in prokaryotes, but also due to the dual taxonomic situation with *Cyanobacteria* in general. From the standpoint of Bacteriological Code of Nomenclature *Cyanobacteria* are considered as prokaryotic microorganisms, but historically they are also botanical objects of study and algologists classify them differently - in terms of the Code of Botanical Nomenclature. In «Bergey's Manual» *Cyanobacteria* with unbranched trichomes and undifferentiated cells are placed in Subsection III (formerly *Oscillatoriales*) consisting of "form-genera" *Oscillatoria*, *Leptolyngbya*, *Planktothrix*, *Arthrospira*, *Limnothrix*, *Borzia*, *Pseudanabaena*, *Geitlerinema*, *Spirulina*, *Lyngbya*, *Crinalium*, *Starria*, *Microcoleus*, *Trichodesmium*, *Tychonema*, *Symploca*, *Prochlorothrix* [1].

In the case of Subsection III classification, situation is complicated by the fact that taxonomically significant features of trichomes such as the presence/absence of sheaths, gas vesicles or weak spiralization are not permanent and may disappear or change during laboratory cultivation. In particular, especially confusing is the classification of morphologically similar cyanobacteria so-called "LPP- group" (named after the first letters of the genera *Lyngbya*, *Plectonema*, *Phormidium* [2]. Botanical genus *Phormidium* (like genus *Plectonema*) has no official status in «Bergey's Manual» and as shown [3, 4] is compound. Thus, to determine unambiguously the taxonomic status of *Cyanobacteria* we should apply a molecular-genetic approach and take into account not only a set of morphological and chemotaxonomical features. Among the molecular-genetic features, analysis of 16S rRNA gene sequences plays a major role. We analyzed 30 representatives from 10 genera of *Cyanobacteria* from Subsection III in the points of their morphology, phylogeny of partially sequenced 16S rRNA genes, RFLP-analysis of 16S rRNA genes and whole genome DNA-fingerprint implemented with specific primers to Hip1 (Highly Iterated Palindrome) sequences.

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Abstracts

Session 2 – Chemotaxonomy *in vitro* vs *in vivo*

THE UPS AND DOWNS OF CHEMOTAXONOMIC ANALYSIS FOR BACTERIAL SYSTEMATICS

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The analysis of chemotaxonomic parameters is now a part of the description of new species of bacteria and archaea. These characteristics generally involve the analysis of the fatty acid composition in bacteria, polar lipid profiles, respiratory quinones as well as other apolar components, such as mycolic acids, when required. The fatty acid composition is generally determined using one widespread semi-automatic gas chromatographic system, followed in some cases by mass spectroscopic analysis. Polar lipid analysis is generally resolved with two dimensional thin-layer chromatography, while respiratory quinones are identified by high performance liquid chromatography. Despite the time and effort necessary for determining chemotaxonomic parameters, these provide a wealth of information on the relationships of new organisms to their closely related species.

However, care must be taken to ensure that these characteristics reflect the relationships of the organisms that are being studied. Extreme care must be taken to produce the best growth medium, aeration, growth temperature that will affect the results to a large extent. This means that several taxa should be compared in the same laboratory under the same conditions. For example, chemotaxonomic characteristics of closely related organisms with different growth temperatures should, be performed at, as common, a growth temperature as possible. Moreover, one should be mindful of misidentified fatty acids, for example, that are not found in the databases of the supplier of the equipment used. Unknown fatty acids and respiratory quinones should be identified by mass spectroscopy whenever possible and especially if they are major components of the organisms.

THE DUMPING GROUND *ARTHROBACTER*: PHYLOGENY AND CHEMOTAXONOMY

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The genus *Arthrobacter* was described by Conn & Dimmick (1947) with three species of which only the type species of the genus, *Arthrobacter globiformis* is still a member of this taxon. In the following years more than 60 novel species of the genus have been proposed. Phylogenetic studies have demonstrated that the genus is not monophlyletic but mixed up with other members of the family *Micrococcaceae*. Also chemotaxonomic data demonstrate significant heterogeneity among members of the genus. The majority of species is characterized by a quinone system with the major mono-saturated menaquinone MK-9(H_2) but also species with menaquinone MK-8(H_2) or completely unsaturated menaquinones such as MK-8, MK-9 or MK-10 have been reported. Like in all other members of the *Micrococcaceae* the peptidoglycan of all *Arthrobacter* species is characterized by the diagnostic diamino acid l-lysine but significant differences occur in the amino acid composition of the interpeptide bridge. At least ten different amino acid compositions of the interpeptide bridges of *Arthrobacter* species have been reported. Furthermore also differences in the polar lipid compositions are known regarding presence/absence of phosphatidlyinositol and presence of certain glycolipids. Interestingly, several phylogenetic groupings within the genus are well reflected by differentiating chemotaxonomic traits. In this contribution the chemotaxonomic history of the genus *Arthrobacter* will be shortly outlined, present knowledge of the distribution of chemotaxonomic traits and their correlation to phylogenetic lines within the genus discussed and conclusions for the taxonomy of the genus drawn.

IN SILICO: RECONCILING COMPUTER CONJECTURES WITH FACTS

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Genomics is transforming our view of the microbial world, and enabling new approaches for analyzing organisms and microbial communities. Given the volume of data, an ever increasing fraction of the genomes are annotated in an entirely automated manner, projecting existing annotations from similar sequences that were annotated in the past. It is commonly pointed out that existing errors in the annotations are relentlessly propagated. Of at least equal importance, the biological diversity of the new genomes is not reflected in the relatively few well-curated genomes from which annotations are being projected. It is not much of a stretch to say that new bacterial genomes are forced into a mold that is shaped like *Escherichia coli*, *Bacillus subtilis*, or one of a few other Bacteria. I will present some preliminary attempts to reconcile genome annotations with characterized properties of the corresponding organisms. Obviously this is easiest when the different phenotypes are defined by different gene sets. However, it appears that accurate projection to additional genomes may be possible even when closely related genes distinguish the alternatives. The most commonly encountered obstacles are not knowing the genes responsible for the phenotype, and the labor involved in collecting the phenotypes from the literature.

MLSA AND POLAR LIPID PROFILE APPLIED TO SPECIES DELINEATION IN THE GENUS *HALORUBRUM*

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Extremely halophilic aerobic archaea, also known as haloarchaea, belong to the family *Halobacteriaceae* within the order *Halobacteriales*, phylum *Euryarchaeota*. Among the genera comprising this family, the largest one is the genus *Halorubrum*, currently including 25 validly described species names. Up to date, new species descriptions in this genus are based on a polyphasic approach, which encompass a phenotypic, genotypic and phylogenetic characterization, mainly using the 16S rRNA gene as molecular marker. However, this gene shows several drawbacks for its use in haloarchaea, such as its low evolutionary rate to reliable discriminate at species level and the frequent lateral gene transfer events occurring among closed relatives. On the other hand, multilocus sequence analysis (MLSA) has been proved to be a rapid and efficient tool to differentiate individual strains and to properly classify them at any taxonomic level within the order *Halobacteriales* [1]

In this study we have carried out a MLSA including the type strains of the species of the genus *Halorubrum*, as well as a total of 28 strains isolated from the hypersaline lake Aran-Bigdol (Iran) initially assigned to this genus. The following five housekeeping genes were analyzed: *atpB*, *EF-2*, *glnA*, *ppsA* and *rpoB'*. The sequences obtained were used to construct individual and concatenated gene phylogenetic trees, by using different algorithms. Although each of the individual genes shows a different evolutionary history, the concatenation of all of them let us to clearly differentiate species, demonstrating in addition that the genus *Halorubrum* remains as a monophyletic group of microorganisms. Besides, our results indicate that some of the strains isolated from the lake Aran-Bigdol clustered with described species of the genus *Halorubrum*, but moreover three additional independent groups can be observed, which could constitute three novel species within this genus.

Furthermore, the polar lipid composition of the new isolated was carefully studied and compared to that of the existing *Halorubrum* species. The main polar lipids found were phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulphate, phosphatidylglycerol and diphosphatidylglycerol. The obtained lipid profiles also supported the new three well-defined phylogenetic groups within this genus. Finally, DNA-DNA hybridization (DDH) studies were performed to determinate if the strains included in those three groups according to MLSA constituted new separated species, and the correlation among MLSA, polar lipid profiles and DDH data were assessed.

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Abstracts

Session 3 – Microbial Systematics in the Classroom

Microbial Systematics in the Classroom. Stewardship of Taxonomy for the 21st Century.

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It is not surprising that PIs and students alike are enamoured by molecular methods, as a huge amount of data can be obtained within a relatively short time. Indeed the application of these methods such as PCR combined with 16S rRNA gene sequencing has revolutionized how microbiologists view microbial diversity and taxonomy. Add to that the sequencing of entire genomes is now becoming routine thus providing another powerful tool in our attempts to identify and classify the enormous amount of diversity throughout the world. As exciting as these developments are, microbes are far more than a collection of genes. To truly understand individual organisms and their interaction within their ecosystem, a polyphasic approach to determine their overall physiology must be undertaken. In searching the literature is it obvious that present-day investigations into microbial diversity are biased strongly towards molecular methods. Fewer laboratories now focus on isolation, cultivation and classical characterization methods. It may be ironic that the very molecular methods that enticed individuals away from classical methods are now driving a return to an interest in microbial systematics. The enormous numbers of "not-yet-cultured' taxa and the lack of skilled professional taxonomists has resulted in only a relatively small number of novel organisms being described. It is the duty of researchers and instructors alike to foster the interest and enthusiasm of students so they may be encouraged to pursue systematics as a viable career option. The classroom and associated laboratories are therefore essential in these early career steps; we the instructors must make the subject of "systematics' accessible and exciting to future generations. The subject of today's presentation will be our approach at the University of Oklahoma and our program of studies. Its foundation is built upon its "Capstone in Microbiology" laboratory class that has been taught for over 20 years. This classroom experience is based on the "van Niel" approach with students isolating and characterizing organisms from enrichments, followed by a polyphasic taxonomic approach employing classical, phylogenetic and chemotaxonomic methods [1]. In addition to the Capstone curriculum other classes and approaches will be discussed.

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SHAPING THE FUTURE OF TAXONOMY THROUGH LEARNING, TEACHING AND ASSESSMENT

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It is still thought that circumscription of microorganisms is not needed, the techniques used are antiquated and that reclassification of organisms is irritating and non-essential. These misconceptions have led to systematics becoming an unpopular scientific niche and a dying art. In fact, the use of current techniques for the description of novel taxa enables their correct identification in the future. This scientific discipline is still needed, especially in the genomic era. However the future is dependent on attracting young scientists to this field of research. Academia is the perfect route for enthusing young scientists about systematics, especially with research led teaching being the main focus of higher education, but this can be through not just final year projects but throughout the course of their degree programme. Innovative and applied approaches to the teaching of microbial systematics compound the core knowledge that is required, but also demonstrate the novel approaches used. Unique modes of assessment direct the students to primary literature, steering students to be more strategic and deep learners. This in turn will hopefully lead to young scientists towards wanting to pursue a research career in taxonomy.

THE MICROBIAL DIVERSTIY ASSESSMENT CLUB FOR LEARNING SYSTEMATICS IN THE CLASSROOM

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The Microbial Culture Collection (MCC) is a recently established Designated National Repository and an International Depositary Authority in India. Realizing the lack of well-trained microbial systematists in todays 'omics' era, MCC took the responsibility of training the future workforce right in the classroom. The Microbial Diversity Assessment Club (MDAC) was envisaged by MCC with support from Dept. of Biotechnology, Govt. of India as part of a long-term objective to create a hands-on learning platform for school and college students across the nation about exploration of microbial diversity and its systematics. In its first phase, MDAC was launched by MCC in 2013 in its parent city, Pune, across all colleges that teach undergraduate and master course in Microbiology. Currently, MDAC involves eight colleges with 64 students. Strategically, each of these colleges have been involved in exploring the diversity of microbes in a specific ecosystem for specific group of taxa. To begin with, only soil from Western Ghats and nearby rivers have been selected. MCC conducts regular sampling tours wherein it provides all financial assistance and supplies for the project and the participating colleges nominate students to this tour for collection of samples. The samples are processed by these students for cultivation on 35 different media under the supervision of their respective college faculty who have been trained at MCC for the very purpose. While conventional microbiological tests are conducted at the college, molecular methods used in systematics of microbes are carried out at MCC by its staff. The students involved in MDAC regularly visit MCC facility for demonstration of these methods. The results are then used by the students to identify, classify and nomenclature of the isolates. So far, 600 cultures have been isolated and are in process for 16S rRNA gene sequence based identification. From the 100 soil isolates sequenced so far, the genera identified are *Arthrobacter*, *Bacillus*, *Microbacterium*, *Paenibacillus*, *Pseudomonas*, *Staphylococcus* and *Streptococcus*. All isolates from the MDAC exploration are preserved at MCC and will be further screened for various bioactive compounds. In its next phase, the MDAC exploration will be expanded statewide. By imparting a hands-on experience of microbial systematics to students in the classroom at an early age, MCC is playing a key role as a biological resource centre in preparing well-trained microbial systematists for the future [1].

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Session 4 – Lessons for systematics from metagenomic studies

HOW MANY SPECIES ARE OUT THERE? BALANCES AFTER ALMOST 40 YEARS OF USE OF 16S RRNA GENE SEQUENCE IN PROKARYOTE SYSTEMATICS

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In the second decade of the XXI century, exemplifying the benefits of the use of the 16S rRNA gene sequence is a commonplace that actually does not need further discussions. Its use has been so well accepted for taxonomists and molecular microbial ecologists that the gene of this molecule is the one with most entries in public sequence repositories. Since the early beginning of this century, its use in new taxa classifications has been regarded as compulsory to reveal their genealogical position. Due to this reason, most of the entries that correspond to taxonomic papers are of good to high quality in terms of length and ambiguities. However, entries corresponding to environmental sequences are often of bad quality or too short for adequate phylogenetic reconstructions. Moreover, during the last two decades, the deposit of environmental 16S rRNA gene entries has increased exponentially, overtaking in orders of magnitude the arithmetic increase of entries corresponding to cultured organisms. To provide to the taxonomist's community the LTP was created as a curated database with prealigned sequences only from type strains of validly published names, and using the universal alignment and format provided by the ARB package [1]. This tool has been useful to calculate the different category thresholds for the taxonomic hierarchy based on the deposited sequences. These thresholds show how consistently taxonomists create their units. In addition, the thresholds calculated had been used to evaluate (i) the correspondence with partial stretches of the molecule, and (ii) the putative abundances of different taxa among the environmental sequences. Finally, we propose a nomeclatural schema for sequences corresponding to uncultured organisms as well as we update the putative census of prokaryotic species in the biosphere.

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"MICROBIAL DARK MATTER" GENOMES FROM GEOTHERMAL SPRINGS: PROGRESS REPORT AND TAXONOMIC PERSPECTIVE

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Although there is no doubt that new species of cultivated genera hide fantastic secrets, it may be argued that there is no greater mystery in microbiology than the yet-unknown activities of major lineages of microbes that have defied >125 years of cultivation efforts. We have recently made significant progress on this so-called "microbial dark matter" problem by sequencing, assembling, annotating, and interpreting genomes from DNA harvested from natural environments (metagenomics) or amplified from single cells (single-cell genomics) [1,2]. Work in a single spring in the U.S. Great Basin, Great Boiling Spring, has yielded the first significant genomic coverage of several candidate phyla of Bacteria, including the 'Atribacteria' (OP9), 'Fervidibacteria' (OctSpA1-106), 'Calescamantes' (EM19), and EM3. Carefully groomed genomic data provide a foundation to address relationships between candidate phyla and known bacterial phyla (lack of formal status in ICSP notwithstanding) and to predict metabolic capacity and structural features. However, creative studies are needed to extend our knowledge of these organisms beyond disjunct reports of 16S rRNA genes and static genomes. Some perspective on the relationship between this type of work and the existing formal taxonomic framework will be provided.

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METAGENOMIC STUDIES ON HYPERSALINE HABITATS AND THEIR IMPACT ON THE SYSTEMATICS OF HALOPHILES

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Hypersaline environments are widely distributed habitats represented by a variety of aquatic and terrestrial systems. Most microbiological studies in these extreme environments have been carried out on aquatic habitats, especially on saline lakes and marine salterns, which are used for the commercial production of salt by evaporation of saeawater. Salterns are excellent models for studying the microbial communities along the salinity gradient, from seawater to salt saturation. Recently, we have carried out metagenomic studies on salterns located in Santa Pola, Alicante, and Isla Cristina, Huelva, Spain. Metagenomic datasets (designated as SS13, SS19, SS33, SS37 and IC21) from ponds with 13 %, 19 %, 33 % and 37 % total salts from Santa Pola saltern and from a pond with 21 % total salts from Isla Cristina saltern, respectively, have been obtained.

The analyses of these metagenomes permitted determining in detail the prokaryotic phylogenomic and metabolic diversity of these hypersaline habitats. As expected, the most concentrated ponds (crystallizers) were dominated by the square haloarchaeon *Haloquadratum walsbyi* and the halophilic bacterium *Salinibacter*, member of the phylum *Bacteroidetes*. However, a higher diversity was determined in intermediate salinity ponds; one of the most abundant microorganisms on these intermediate salinities was a member of the *Gammaproteobacteria*, most closely related to the genus *Alkalilimnicola*. We used several strategies and culture media in order to isolate this organism in pure culture. We report the isolation and taxonomic characterization of this new, never before cultured microorganism, designated M19-40, using a medium with a mixture of 15 % salts, yeast extract and pyruvic acid as carbon source. Morphologically it has a unique shape, from small curved cells (young cultures) to long spiral cells (older cultures). Is a Gram-negative, non-motile bacterium, strictly aerobic, non-endospore-forming and heterotrophic. Moderately halophilic, able to grow at 10-25 % (w/v) NaCl, with optimal growth at 15 % (w/v) NaCl. This bacterium is very abundant in intermediate salinity ponds, according to the genomic data, but is absent in seawater and the crystallizer saltern ponds. The features of this new bacterium showed that it constituted a new genus and species, for which the name *Spiribacter salinus* gen. nov., sp. nov. is proposed. Other new halophilic bacteria and archaea that may constitute new taxa and probably are abundant microbes of these habitats are under investigation.

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Session 5 – New approaches and new taxa

EzGENOME: A GENOME DATABASE FOR ACCURATE TAXONOMIC IDENTIFICATION OF PROKARYOTES BASED ON GENOME SEQUENCES

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The rapid development of sequencing technology has fundamentally changed the view of genomics. In particular, next generation sequencing platforms have greatly accelerated the production of prokaryotic genome sequences at an unflagging rate for the last few years, which has extended our knowledge of biology, physiology, and evolution of microbes. However, there have been a growing number of genomes which harbor inaccurate taxonomic information as more genome sequences are available. The erroneous taxonomic information may lead to the wrong conclusion in many further genomic researches. Here we report the EzGenome database (available online at http://www.ezbiocloud.net/ezgenome/), which contains both draft and complete genome sequences from publicly available prokaryotic genome databases. One of the prominent features of the database is that all collected genomes were reclassified more accurately based on taxonomic marker genes (i.e. 16S rRNA) and average nucleotide identity (ANI). This database provides ANI dendrograms at the family, genus, and species level. Furthermore the database offers useful basic genome's attributes such as genome-based GC ratio, size, CDS, etc. and tools for additional genomic researches.

CVTREE3: AN EFFECTIVE GENOME-BASED AND ALIGNMENT-FREE PHYLOGENETIC TOOL WITH INTERACTIVE TREE DISPLAY AND TAXONOMIC COMPARISON

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CVTree constructs whole-genome based phylogenetic trees without sequence alignment by using a Composition Vector (CV) approach. It was first developed to infer evolutionary relatedness of Bacteria and Archaea and then successfully applied to viruses, chloroplasts, and fungi. In this talk, after briefly introducing the methodology, I will describe the newest CVTree3 web server (http://tlife.fudan.edu.cn/cvtree3). Prokaryote phylogeny is verified by direct comparison with taxonomy at all ranks from phyla down to species. An interactive tree-viewer allows searching, collapsing, and expanding the tree branches. An automatic generated list of taxa, which are monophyletic or not, helps to comprehend the overall agreement of phylogeny with taxonomy and hints on possible taxonomic revisions. We hope CVTree3 will become a convenient tool in the hands of microbiologists.

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THREE-DIMENSIONAL GRAPH ANALYSIS OF PROKARYOTIC AND EUKARYOTIC GROUPS AND MATHEMATICAL MODEL OF EVOLUTION OF LIFE

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This presentation covers the problems of microbial diversity and evolution indicated in the symposium's title from the point of view "Undiscovered Taxa; New Approaches" since it discuss an evolutionary development of known taxa according to the geometric coordinates by certain mathematical function. This geometrical function may indicate the coordinates of extinct or existing and yet undiscovered taxa.

Previous analysis of valid species of Archaea demonstrated their specific distribution in two-dimensional diagrams: pH/T, T/Salinity & pH/Salinity [1]. The position of Archaean species located at the top corner of the diagram, above the graph-crossing diagonal; As a result, the Rules of the Diagonal were formulated & described. Three-dimensional analysis of Archaea in logarithmic scale showed the distribution of Archaea in a function of a hyperboloid; for Halobacteria - Crenarchaeota – two sheet hyperboloid, and for all groups – one sheet hyperboloid. It was shown that the hyperboloid has an inclination of 67.5 ^{o}. Constructed two-dimensional graph of the hyperbola, clearly confirmed 3D function of a hyperboloid; the symmetry of asymptotes evidently explained the inclination of the hyperboloid in 3D. Furthermore, geometrically, involvement of other biological groups was shown [2]. Following step was to analyze all biological groups to demonstrate the geometry of their eco-physiological coordinates to outline a total, complete model of the evolution of life on Earth [3].
For microbiologists, suggested mathematical model may indicate precise regions of temperature, pH, and salinity that should be examined with molecular probes on the subject of DNA of novel species. The majority of samples from Earth's ecosystems have been studied for microbial diversity, but the only narrow spectrum of buffer systems was checked during attempts to isolate new microorganisms.

In this article, we demonstrate geometry of Prokaryotic and Eukaryotic groups in co-evolution and possible interaction, discuss some critical changes of geometry for separate taxonomic groups during the process of evolution, and focus on a possible involvement of mathematical laws and application of geometric function for explanation of the evolution.

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MALDI-TOF MS, A TRANSFORMATIVE ANALYTICAL TOOL FOR THE CHARACTERISATION AND AUTHENTICATION OF MICROORGANISMS

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Mass Spectrometry (MS) has long been used as a powerful analytical tool for microbial systematics and data derived incorporated into numerous species descriptions. This began with the analysis of lipids such as respiratory quinones and cellular fatty acids by MS or GS-MS. Subsequently, Pyrolysis-MS and Fast Atom Bombardment-MS were introduced but these approaches failed to gain a foothold in microbiology. By contrast, MALDI-TOF MS has emerged as a formidable, transformative diagnostic tool for the characterisation of microorganisms.

Described as a quantum leap for clinical microbiology, the use of MALDI-TOF MS for microbial taxonomy has lagged behind. Because the technique profiles the cell's stable ribosomal protein content, we established the first comprehensive database of profiles [Keys et al. (2004) Infect. Gen. Evol,**4**,221-242] and now utilise it extensively for the characterisation of strains held in the National Collection of Type Cultures (NCTC), a UK-based culture collection with over 5000 bacterial strains of medical importance.

Data to date indicate that for many taxa, it currently surpasses the resolution of 16S rRNA and heterogeneity among several species can be discerned. Many fastidious anaerobic taxa for example, that hitherto lacked discriminative characters and were poorly resolved, are being confidently identified for the first time. Consequently, there has been a resurgence of interest in anaerobic microbiology. NCTC now employs the technique, as part of a polyphasic approach for strain authentication.

PROBLEMS OF DESCRIBING BACTERIA FROM RARELY CULTURED PHYLA

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Significant breakthroughs in cultivation of bacteria from poorly studied and as-yet-uncultivated phylogenetic groups have been made during the last decade. However, in contrast to the growing number of reports on isolation of various elusive microbes, the taxonomically described diversity within rarely cultured bacterial groups remains very poor. Two good examples are the cosmopolitan phyla *Acidobacteria* and *Planctomycetes*. The number of validly described species in each of these phyla hardly exceeds two dozens, although a few hundreds of 16S rRNA gene sequences in the GenBank are annotated as belonging to isolates of planctomycetes and acidobacteria. How did it happen that these unique isolates remained uncharacterized? One of the main reasons is that most planctomycetes and acidobacteria are slow-growing organisms that are difficult to manipulate in the laboratory. Many routine procedures, such as collecting the biomass for enzyme assays, chemotaxonomic analyses or DNA:DNA hybridization experiments become an endless torture for the microbiologist. Growth experiments may also represent a difficult task since many planctomycetes and acidobacteria do not grow homogenously but form large flocks and aggregates. Nearly all standard assays that are widely used for characterizing metabolic capabilities of the examined strains were originally developed for fast-responding microorganisms; these assays are poorly applicable for slow-growing bacteria. Finally, the chemotaxonomic analysis becomes a highly challenging task because many planctomycetes and acidobacteria contain unique lipids, which cannot be identified using the routine TLC technique. In summary, describing unusual microbes requires adjusting not only the commonly accepted, routine approaches but also the views on describing novel taxa.

IS THERE STILL ANY ROOM FOR IMPROVEMENT ON THE SYSTEMATICS OF GENERA OF THE FAMILY *MICROMONOSPORACEAE?*

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Members of the genera *Micromonospora* and *Salinispora* follow a complex life cycle which normally involves the presence of substrate or vegetative mycelia and sporulation with single spores born on the vegetative hyphae followed by the synthesis of a dark extracellular polysaccharide. Bergey's Manual states that micromonosporae rarely produces aerial mycelia (AM) and if so, is considered "*sterile*" [1] while there are no reports of MA in *Salinispora*, the first actinobacterial genus of marine origin described [2]. During the characterisation of novel isolates assigned to the family *Micromonosporaceae* from the Sea of Cortes [3], it was observed that AM is produced reproducibly in the presence of certain carbon and/or nitrogen sources by micromonosporae and a putative novel genus related to *Micromonospora* and *Salinispora*. Micromanipulation of the AM subcultured onto fresh media produced colonies; hence, this structure should not be called "*sterile*". This would be the first report of the presence of "inducible" AM in micromonosporae and suggests that there is still room for improvement on the circumscription of the genus *Micromonospora* and of taxonomic value to related genera within the family *Micromonosporaceae*.

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A NEW POLYPHASIC APROACH FOR THE CHARACTERISATION OF THE FISH PATHOGEN *FRANCISELLA NOATUNENSIS*; A BASELINE FOR A PROPOSAL OF MINIMAL STANDARDS WITHIN THE GENUS FRANCISELLA

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Members of the genus *Francisella,* family *Francisellaceae* order *Thiotrichales* class *Gammaprotobacteria* phylum *Proteobacteria* are Gram negative, non-motile, non-sporulating, aerobic, coccobacilli prokaryotes (size 0.2-1.7 µm) with a wide geographical and ecological distribution [1]. With the exception of *Francisella guangzhouensis* all members with a binomial name are either obligate or facultative endosymbionts of eukaryotic cells and most have the ability to cause severe disease in their hosts. *Francisella noatunensis* is one of the most recently recognized species, and is the causative agent of the emerging disease "Piscine Francisellosis", recently reported worldwide affecting several fresh water and marine fishes in warm and cold water conditions [2]. Since first described, the nomenclature of *F. noatunensis* has been under constant rearrangement, this mainly due to their fastidious nature that complicates their primary isolation and subsequent characterisation. Here we described a polyphasic approach for the characterisation of novel and archived strains of *F. noatunensis*. Ecological, phenotypical, serological, chemotaxonomical, genetical and genomic analyses were performed compiling methodologies used in the description of the currently valid *Francisella* species and subspecies. The results of this study indicate the necessity of a taxonomical rearrangement where a new combination within the genus is prosed and highlight the need for "Minimal Standards" at least for the description of taxa below the genus level. In order to compare and discuss these results, a brief historic review of the intricate and complex taxonomy of *Francisellaceae* will also be presented.

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NOVEL STREPTOMYCETES RELATED TO THE *STREPTOMYCES ACIDISCABIES* SUBCLADE

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Potato Common Scab is an important worldwide disease caused mainly by streptomycetes. For many years *Streptomyces scabies* was considered the main causal agent of common scab and, therefore, was the first phytopatogenic species to be described [1]. Later, eleven species have been validly described as pathogenic scabby species, including *S. acidiscabies* [2]. In the present study, nineteen putative strains of *Streptomyces* were isolated from potatoes with visible common scab lesions. The samples were from Ahome, Los Mochis, Sinaloa and the isolates characterised by combining phenotypic, genotypic and *in vitro* and *in vivo* assays. All the isolates were found to contain LL-diaminopimelic acid in their cell wall and showed different fingerprinting patterns after BOX-PCR. Phenotypically, the strains showed a wide range of spore mass colour on ISP media and typical straight or spiral spore chains of smooth to irregular ornamentations. Production of thaxtomin was also detected for some of the isolates using analytical methods. A phylogenetic study showed that the isolates belong to the genus *Streptomyces*, five of them are associated to the *S. acidiscabies* subclade and may well represent new species, an observation supported by both the geno- and phenotypic properties. To our knowledge, this is the first comprehensive study to establish the identity of the potato common scab agent in Mexico and suggests that such agents may well be currently underspeciated.

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Poster Abstracts

A NOVEL THERMOPHILIC, ANAEROBIC SACCHAROLYTIC BACTERIUM ISOLATED FROM AN ANAEROBIC BATCH DIGESTER TREATING ANIMAL MANURE AND RICE STRAW

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A thermophilic, anaerobic, fermentative bacterium, strain A6^T, was isolated from an anaerobic batch digester treating animal manure and rice straw in China. Strain A6^T stained Gram-positive, and was non-motile. Cells were slightly curved rod, with a size of 0.6–1 μm x 2.5–8.2 μm. Terminal spores were produced. The temperature range for growth was 40–60℃, with optimum growth at 50–55 ℃. The pH range for growth was 6.5–9.0, with optimum growth at pH8.5. The NaCl concentration range for growth was 0–15 g/L, with optimum growth in the absence of NaCl. Yeast extract was required for growth. Glucose, maltose, xylose, galactose, fructose, ribose, lactose, mannose, raffinose, sucrose, arabinose, cellobiose and yeast extract were used as carbon and energy sources. The main fermentation products from glucose were ethanol, acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, H_2 and CO_2. Thiosulfate was used as electron acceptors. The G+C content of the genomic DNA was 36.6 mol%. The predominant fatty acids were $C_{16:0}$ (30.60%), iso-$C_{17:1}$ (30.3%), $C_{14:0}$ (18.1%), $C_{16:1}$ w7c (5.6%). Respiratory quinones were not detected. The polar lipid profile comprised phosphoglycolipids, phospholipids, glycolipids, one diphosphatidylglycerol, one phosphatidylglycerol and one unidentified lipid. Phylogenetic analyses of the 16S rRNA gene sequence indicated that the strain was closely related to *Defluviitalea saccharophila*(=DSM 22681^T) with similarity of 96.0%. Based on the morphological, physiological and taxonomic characterization, strain A6^T was proposed as a novel species of genus *Defluviitalea*. The type strain is A6^T (=DSM 28090^T).

FERRUGINIBACTER PROFUNDA SP. NOV., NOVEL MEMBERS OF THE FAMILY *CHITINOPHAGACEAE*, ISOLATED FROM DEEP FRESHWATER SEDIMENT OF A RESERVOIR

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Strain DS48-5-3^T was isolated from a 48M deep sediment sample taken from Daechung Reservoir, Republic of Korea. Comparative 16S rRNA gene sequence studies showed a clear affiliation of this strain into the phylum *Bacteroidetes*, which was most closely related to *Ferruginibacter alkalilentus* HU1-GD23^T and *Ferruginibacter lapsinanis* HU1-HG42^T, showing 16S rRNA gene sequence similarities to the type strains of these species of 96.0% - 96.5% similarity. Cells were Gram-negative, aerobic, non-motile, and rod-shaped. The predominant ubiquinone was MK-7. The major fatty acids were iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, and iso-$C_{15:1}$ G. The G+C content of the genomic DNA of the strain DS48-5-3^T was 40.2 %. On the basis of polyphasic evidences, it is proposed that strain DS48-5-3^T should belong to a novel species, for which the name *Ferruginibacter profunda* sp. nov. (type strain DS48-5-3^T =KCTC 32478^T =JCM 19431^T), is proposed.

ACTINOBACTERIAL DIVERSITY IN HIGH ALTITUDE ATACAMA DESERT SOILS AND REGOLITHS AND ITS BIOTECHNOLOGICAL POTENTIAL

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Our strategy for successful biodiversity campaigns rests on the premise that extreme biomes are likely to contain novel actinobacteria with the capacity to produce novel bioactive compounds relevant for improved healthcare[1]. This approach to drug discovery is proving to be effective when applied to hyper- and extreme hyper-arid Atacama Desert soils and regoliths[2]. In the present study this taxonomic approach to drug discovery was used to selectively isolate, dereplicate and screen filamentous actinobacteria found at high altitude sites (up to 5000 m) in the Atacama Desert. Novel / putatively novel filamentous actinobacteria, notably streptomycetes, isolated from surface and subsurface samples from the Chajnantor plateau near the ALMA observatory were found to produce a broad range of bioactive compounds in plug assays, some of which were shown to be novel in LC-MS analyses thereby providing further evidence of a coupling between taxonomic and chemical diversity.

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TAXONOMIC DIVERSITY OF ACIDOPHILIC ACTINOBACTERIA AS A ROADMAP TO DRUG DISCOVERY

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Filamentous actinobacteria are the source of almost half of all known natural products and typically have whole-genome sequences which contain > 20 biosynthetic gene clusters that code for known or predicted secondary metabolites[1]. Although clearly an underdeveloped resource, it is difficult to discovery new chemical entities from known actinobacteria as screening them leads to the costly rediscovery of known compounds. Consequently, new strategies are needed to isolate, dereplicate and recognise new taxa for screening purposes. This taxonomic approach to drug discovery was used to selectively isolate and highlight novel acidophilic actinobacteria from litter and mineral horizon of a spruce forest soil. Four novel / putatively novel acidophilic species of *Actinospica*, *Nocardia* and *Streptacidiphilus* were discovered some of which produced new natural products thereby providing further evidence that filamentous actinobacteria from extreme habitats are a potentially rich source of new bioactive compounds for healthcare[2].

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Molecular diversity of bacteria isolated from groundnut (*Arachis hypogaea*) rhizosphere and nodules from rainfed Pothwar, Pakistan

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Groundnut is an important cash crop of Pothwar, and fixes about 10-30 kg N ha^{-1} [1]. Therefore, there is a need to characterize and identify the potential PGP strains from groundnut rhizosphere in order to explore its N_2-fixation potential for increasing crop yield by co-inoculation with *Rhizobium* sp. The bacteria were isolated by adopting the dilution plate technique, using phosphate saline solution as buffer and Tryptic Soy Agar as general media for bacterial growth. For isolation of *Rhizobium sp* Yeast Mannitol Agar was used. The purified strains were screened and characterized for *nif*H gene by using forward and reverse primers primers i.e. $PolF^b$(TGC GAY CCS AAR GCB GAC TC) and $PolR^b$ (ATS GCC ATC ATY TCR CCG GA), Indole Acetic Acid production and P-solubilization. Biochemical characterization of the strains was carried out by using Biolog, API ZYM and API 20E kits. More than 50 bacteria were isolated from groundnut rhizosphere soil (designated as G1, G2, G3,….etc) and nodules (designated as BN1, BN2, BN3,….etc). These bacteria solubilized tricalcium phosphate in the range of 30-300 µg ml^{-1}. Similarly IAA produced by these isolates was in the range of 4-22 µg ml^{-1}. Some isolates carried *nif*H gene (+). DNA of the potential strains was amplified by PCR, using forward and reverse primers: 9F (5-GAGTTTGATCCTGGCTCAG-3) and 1510R (5-GGCTACCTTGTTACGA-3), and confirmed by gel electrophoresis. Comparative analysis of 16S rRNA gene sequence performed by universal primers, confirmed the diversity of rhizobacteria and the isolates belonged (>99% sequence similarity) to genus *Rhizobium*, *Bacillus* and *Chryseobacterium* etc. Four *Rhizobium* sp. (*R. alkalisoli*, *R. massiliae*, *R. loessense* and *R. huautlense*) were isolated from nodules and their nodulation test was performed to check the nodule formation on host plant. Housekeeping genes analysis was performed for five housekeeping genes *atpD*, *recA*, *rpoB*, *dnak* and *glnII* to confirm the taxonomic status of isolated *Rhizobium* species. One *Rhizobium sp.* (BN-19) exhibited the 16S rRNA sequence similarity of 97.5% with its closely related defined *Rhizobium* species. For all five housekeeping genes, the similarity of BN-19 was less than 90% with all defined species. DNA-DNA hybridization with its closely related reference strains confirmed the novel status of the candidate BN-19. Phylogenetic tree based on neighbor joining method were constructed by retrieving their closest relatives through BLAST search using MEGA4 software. These potential PGPR and *Rhizobium* will be further studied to be used as bio-innoculant for crop production.

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THE GUT MICROBIOTA OF POLLINATORS: AN UNKNOWN AND UNEXPLORED TREASURE CHEST OF BIODIVERSITY

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Bumblebees are important pollinators of tomatoes, sweet pepper and many other commercial crops and wild plants. However there is currently great concern about their worldwide decline and that of other pollinators like butterflies and hoverflies [1]. These declines may have a detrimental economical impact and may create an instable ecosystem. The gut microbiota of bumblebees consist of few and very specific bacterial species [2], [3] and include *Snodgrassella alvi*, *Gilliamella apicola* and *Bifidobacterium bombi*. These symbiotic gut bacteria may contribute to the health of bees by helping with the digestion of pollen, the detoxification of compounds and the pathogen inhibition [4]. Therefore an inventarization of the cultivable bacteria in the gut of bumblebees and of their functionality is being made.
We will present polyphasic taxonomic and whole genome sequence data reporting the presence of novel bacteria belonging to the genera *Gilliamella*, *Bifidobacterium*, *Leuconostoc* and *Lactobacillus*, and to the family *Acetobacteraceae*.

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A NEW THERMOPHILIC SPECIES OF THE GENUS *RUBROBACTER*, FROM A GEOTHERMAL SPRING IN ALASKA

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Four isolates, designated, MD2-16^T, MD2-17, MD2-18 and MD2-19 were isolated on Marine Agar from a geothermal hot spring at Chena, Alaska. Phylogenetic analysis based on 16S rRNA gene sequence comparisons indicated their membership of the genus *Rubrobacter*. The 16S rRNA gene sequences show highest pairwise similarity at 95.3% to the species *R. radiotolerans*. Colonies are bright pink on *Thermus* medium. The strains are strictly aerobic, catalase and oxidase positive, reduce nitrate to nitrite and are halotolerant. Cells are non-motile, non-spore forming pleomorphic rods. Optimal growth was observed at 50 °C, pH 8-9 and 3-4% NaCl.

The major fatty acids of strain MD-16T are $C_{16:0}$, $C_{16:0}$ 12-methyl and $C_{17:0}$ 12 methyl. Polar lipid analysis demonstrated the presence of diphosphatidylglycerol, phosphatidylglycerol, phosphoglycolipid as well as two glycolipids, three phospholipids and an aminolipid. The major menaquinone is MK-8. The mol% G+C content of the DNA of strain MD2-16^T is 66.7. Strain MD2-16^T demonstrated extreme tolerance to desiccation when compared to other species of the genus. Based on the genotypic, phenotypic and biochemical characteristics, we describe a new species: *Rubrobacter chenensis* sp. nov., the type strain of which is MD2-16^T

RELATIONSHIP OF WHOLE GENOME SEQUENCE SIMILARITY TO DNA HYBRIDIZATION IN PROKARYOTES

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In the original proposal of Wayne et al [1] two measures of genetic relatedness were proposed to set the boundary for prokaryotic species. The first was the change in the melting temperature (or ΔTm) of heteroduplex DNA, and the second was the extent of DNA-DNA hybridization (DDH). While this approach was justified given the experimental error inherent in these methods at the time, genomic sequencing has the potential to measure both parameters with great precision [2, 3]. Moreover, theoretical concerns suggest that the relationship between the ΔTm and DDH might vary among different groups of prokaryotes, and a single criterion might now be appropriate. To test this hypothesis, 17 groups of prokaryotes that were represented by complete genomic sequences for strains with a range of DDH values were chosen for further study. These groups included free-living and symbiotic heterotrophs, autotrophs, Bacteria, Archaea, mesophiles, and hyperthermophiles. Based upon their genomic sequences, the DDH was calculated as proposed by Auch et al. [2]. The ANIb, which is a measure of the sequence similarity and comparable to the ΔTm, was calculated as proposed by Richter and Rosselló-Móra [3]. As observed by others, the DDH was linearly correlated to the ANIb when the ANIb was >75%. However, the ratio (100-DDH)/(100-ANIb) varied from about 2.4 to 4.4 or nearly two-fold between different prokaryotic groups ($p<0.001$). Because the DDH and ANIb provide different measures of relatedness, it is no longer appropiate to consider both when delineating species. We recommend that ANIb or other measures of relatedness based upon sequence similarity should be used for delineating species in the future.

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