日本微生物資源学会第 18 回大会要旨

(JSCC Symposia for the 60th Anniversary at IUMS2011 Sapporo)
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JSCC Award Lecture

2011 JSCC Award for Young Scientists

Phylogenetic studies on the bacterial phylum '*Verrucomicrobia*'

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The phylum '*Verrucomicrobia*' (Hedlund *et al*., 1997) is one of the primary lineages within the domain *Bacteria*. A lot of molecular phylogenetic approaches and culture-independent studies based on 16S rRNA gene sequences revealed that the members of this phylogenetic group have been detected in a very wide range of quite different habitats within the global ecosystem. These lineages have been informally classified into five subdivisions numbered 1 to 5 and have also been classified into six monophyletic subdivisions numbered 1 to 6 of which three are recognized in the second edition of *Bergey's Manual of Systematic Bacteriology* as the families *Verrucomicrobiaceae* (subdivision 1), *Opitutaceae* (subdivision 4) and '*Xiphinematobacteriaceae*' (subdivision 2). Since the six informal monophyletic subdivisions of this phylogenetic group were first proposed, the names of only a few species belonging to subdivisions 1 and 4 have been validly published. The class *Opitutae*, comprising two orders: *Puniceicoccales* containing the family *Puniceicoccaceae* and *Opitutales* containing the family *Opitutaceae*, was formally proposed recently for the classification of subdivision 4. However, in spite of wide ecological distribution of representatives of the phylum '*Verrucomicrobia*' in nature, owing to relatively few pure cultivated and characterized species, the classification of this phylum is still ambiguous and informal. For this reason, for formal classification of the phylum '*Verrucomicrobia*', it is recommended that many verrucomicrobia that thrives in a wide range of terrestrial, aquatic and marine habitats should be isolated andtaxonomically investigated. In the present study, we attempted to elucidate the phylogenetic relationships of thirty novel strains isolated from various marine environments using a polyphasic taxonomic approach including 16S rRNA and *gyrB* gene sequence analyses, together with molecular, physiological, biochemical and chemotaxonomic analyses to characterize the novel isolates.

Reference

Hedlund *et al*. 1997. Antonie Van Leeuwenhoek **72**: 29-38.

JSCC Workshop for Practice of Culture Collections

"Databases, Tools and Networks to Promote Microbial Culture Collections and Systematics"

1. Impact of the advancement of information and sequencing technologies to culture collections

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Organisation for Economic Co-operation and Development (OECD) published a report entitled "Biological Resource Centres-underpinning the future of life sciences and biotechnology" in 2001. The report has encouraged microbial culture collections (MCCs) to evolve to microbiological resource centers (MRCs) that meet *the high standards of quality and expertise demanded by theinternational community of scientists and industry for the delivery of biological information andmaterials (OECD report).* Quite a few MCCs have actually conformed ISO standards to claim theircredibility to be MRCs.

In a decade since the publication of the OECD report, information technology (IT) and sequencing technologies (ST) have advanced extensively. The Internet and PC clusters prevail on the globe and cloud computing become popular. In the meantime, so-called next-generation sequencers have made us possible to sequence microbial genomes in a day. In addition, the third and even the fourth generation sequencers are arising. It is hard for anyone to estimate the volume of sequence data produced in a year. Thus, it is ambiguous if IT will be able to meet a need caused by ST. Anyway, MCCs will be able to advance to MRCs, if they wisely utilize the advanced technologies.

In 2010, CBD COP10 in Nagoya adopted "Aichi Targets" for 2020. One of the targets is "*By 2020, knowledge, the science base and technologies relating to biodiversity, its values, functioning, status and trends, and the consequences of its loss, are improved, widely shared and transferred, and applied.* (http://www.cbd.int/sp/targets/)". MRCs are granted a privilege to provideknowledgebase for wide communities thanks to the advanced IT and ST.

2. WFCC-MIRCEN World Data Centre for Microorganisms (WDCM)

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WDCM provids a comprehensive view on culture collections and their holdings to the relevantcommunities. They are now as diverse taxon-wide, application-wide and region-wide as microbesare.

In 1972, Dr. Stanley Morris Martin published the first edition of World Directory of Collections of Cultures of Microorganisms. WDCM was maintained by the University of Queensland since 1972. The WDCM director Professor V.B.D. Skerman, issued the 2nd edition of the World Directory in 1982.

After WDCM was transferred to RIKEN in Japan and directed by Professors Kazuo Komagata in 1986, the World Directory became an on-line database. Professor Hideaki Sugawara took over the directorship when Professor Komagata retired and transferred WDCM to the National Institute of Genetics (NIG) in Japan in 1997. WDCM in NIG started Web sites for both WDCM and WFCC.

WDCM now is managed by a new host, namely, Information Center, Institute of Microbiology, Chinese Academy of Sciences from 2011. The 3rd generation of WDCM in Beijing will expand the personalized services to culture collections and their customers, e.g.

- Customization and mail push of microbial information
- Online collaborative working platform
- Bioinformatics analysis platform
- Website and online catalogue for culture collection centers in need

After the relocation of WDCM, WDCM has worked out the new WFCC webpage and online reference strain catalog. The new WFCC website allows users to submit their own news and pictures to enhance user feedback. WDCM reference strains online catalog will help users find local sources of the reference strains by citing all collections and providing contact details and the collection's unique reference. Furthermore, WDCM is developing a Citations Statistics System of Microbial Resources（CSMR）as one of the very important services pro-

vided to WFCC members, which provides searching and statistics tools for culture collections or strains.

3. The database and network for microbial resources in the Korean National Research Resource Center

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The KNRRC project has started in 1995 by the Ministry of Science and Technology (MOST) which became the Ministry of Education, Science and Technology (MEST). The Korea National Research Resource Center (KNRRC) is consisted of 36 research resource centers (RRCs) located in 25 universities, 5 core centers (human-originated resources, plant, animal, microorganism, and fusion-matter), and a central office. Its collection includes microorganisms, plants, animals, human specimens, and non-biologic materials. The KNRRC head quarters provides a total management system for the RRCs including database management, on-line ordering, guidelines, educational programs, and certification of resources, workers, and RRCs in order to provide Authenticated, Customer-oriented resources with Easy Access (ACE). The KNRRC headquarters is serving as the head office of Asian Network of Research Resource Center (ANRRC) as well as the Asian Chapter of the International Society for Biological and Environmental Repositories (ISBER).

There are twelve BRCs in the field of microbiology. These are Center for Fungal Genetic Resources, Waterborne Virus Bank, Bacteriophage Bank, Culture Collection of Mushrooms, Korea Bank for Pathogenic Viruses, Plant Virus GenBank, Myxobacteria Bank, Korean Lichen & Allied Bioresource Center, Korea Marine Microalgae Culture Center, Culture Collection of Antimicrobial Resistant Microbes, *Helicobacter pylori* Korean Type Culture Collection, and Korea National Environmental Microorganisms Bank.

Microbial BRCs are managing 16 different kinds of microbial resources including virus, bacteria, fungi, mushroom, microalgae, their genome, DNA libraries, cloned genes, antibodies, human serum, and host organisms. Each microorganism or its extract is stored in various forms such as freeze-dried powder under vacuum and frozen state to meet the researcher's need. The total inventory of microbial BRCs is over 54,300 items. Among these, 32,600 items have accession numbers with full information in the DB and open to the public.

In 2010, total management systems were set up for Korea Bank for Pathogenic Viruses, *Helicobacter pylori* Korean Type Culture Collection, Culture Collection of Antimicrobial Resistant Microbes, and Myxobacteria Bank. By the end of 2011, the management systems for Center for Fungal Genetic Resources and Waterborne Virus Bank will be established. The whole system for the twelve microbial BRCs will be completed in 2013.

The total management system includes homepage, DB, and inventory system for each RRC. Information for each resource is consisted of standard data set (SDS) or mandatory data set (MDS) and supplementary data set provided by each RRC. The SDS contains ordinary name, genus, species, registered number in each RRC, numbers of the original resources, the minimum number for storage, availability for distribution, domestic or foreign, name of isolator, location of isolation, isolation date, date and institution of registration, and possible usage. Via inventory system, RRC can efficiently and precisely perform in-house management such as location of resources, distribution data, as well as the numbers of resources left in storage.

4. EzTaxon-e and EzGenome: new tools for prokaryotic systematics

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Next generation sequencing (NGS) provides an effective way of obtaining massive genome data with a reasonable cost. To cope with the flood of data generated by NGS, nucleotide sequence databases with care curation are essential. In this presentation, I will introduce two web-based databases, namely EzTaxon-e and EzGenome. EzTaxon-e database (http://eztaxon-e.ezbiocloud.net/) is an extension of the original EzTaxon database which holds 16S rRNA sequences of type strains of validly named species. In EzTaxon-e, representative sequences in public domain that do not represent validly name species are carefully selected and compiled. In addition to ~11,000 sequences associated with validly named species, 24,000 representative sequences were included and named uniquely. EzGenome database (http://ezgenome.ezbiocloud.net/) is a new prokaryotic genome database that is curated manually. All of genomes compiled are identified using EzTaxon-e's hierarchical taxonomic system, and all genes included were annotated using the same manner. Both EzTaxon-e and EzGenome databases are interconnected and should be useful tools for microbiologists.

EzTaxon-e database

The original EzTaxon database (http://www.eztaxon.org) contains 16S rRNA sequences of typestrains of validly described names. In new database, called EzTaxon-e, the followings were included (As of August 15, 2011).

Number of names/sequences	Count
Validly described names	9756
Validly described names (in press)	385
Invalid names	105
Candidatus names	221
Tentative species names proposed in EzTaxon-e*	24201

* Tentative species names proposed in EzTaxon-e were generated to designate representative sequences which were selected to represent a group of sequences defined by 97% sequence similarity. This is newly added feature of new EzTaxon-e database.

Availability: http://eztaxon-e.ezbiocloud.net/

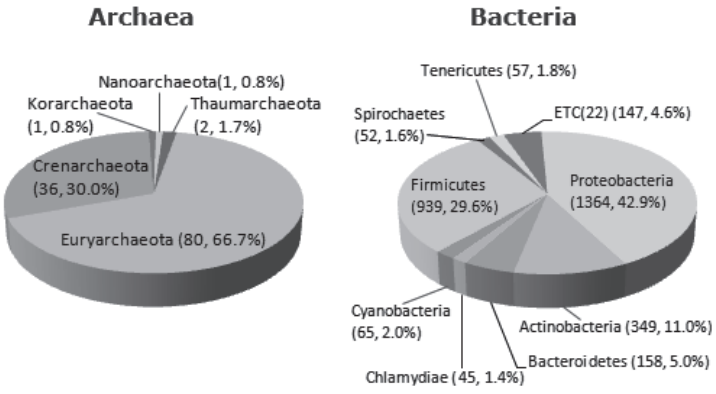
EzGenome database

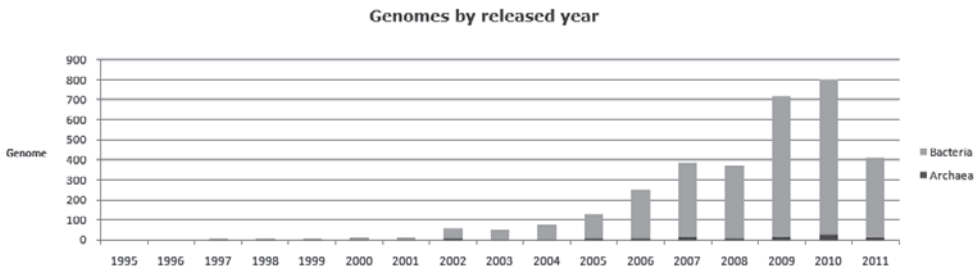
EzGenome is new taxonomy-oriented prokaryotic genome database. The database holds 3,444 (As of August 15, 2011) genomes including 1,698 complete genomes. Most of genomes are of bacteria (3,321 genomes), but 123 archaeal genomes were also included.

In EzGenome database, all genomes were taxonomically re-identified and properly labeled with up-to-date nomenclature.

EzGenome has following functions:

- BLAST search for all genomes (contigs, DNA of CDS, protein of CDS)
- BLAST search for each genome
- Genome Size Predictor: genome size is predicted by homolog search
- Homolog Extractor: Homologs (either DNA or proteins) are extracted from a set of taxon. For example, gyrA gene can be extracted from the family *Enterobacteriaceae*. This function should be useful for multilocus sequence analysis (MLSA) and other phylogenetic analysis.





Availability: http://ezgenome.ezbiocloud.net/

5. Fungal DNA barcoding and related projects at the National Museum of Nature and Science, Japan

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Because of their cryptic nature, the true diversity of microbes can only be well understood with molecular data. Biologists of zoology and botany communities have also realized the importance of universally available molecular markers as "DNA barcodes" and mitochondrial *CO1* and chloroplast *rbcL* and *matK* genes were selected for animals and plants, respectively. It is surprising, however, that one of major microbial groups on the terrestrial ecosystems, i.e., Fungi, do not have an officially claimed DNA barcoding markers yet. Although the animal and fungal kingdoms are sister groups, the application of *CO1* as fungal DNA barode has been quickly rejected. It is well documented that fungal mitochondrial genomes tend to have large and frequent introns, which make designing the universal primers for PCR amplification difficult, if not impossible. On April 2011, a workshop of fungal barcoding was held in Amsterdam, and there reached a unanimous agreement selecting the ITS region of nuclear ribosomal genes as fungal DNA barcode.

This presentation summarizes the current status of fungal DNA barcoding projects, with particular emphasis on ongoing barcode-related projects at the National Museum of Nature and Science, Japan. The projects include:

(1) DNA barcoding from the type specimens of mushrooms;
(2) Epitypification of mushroom specimens described from Japan;
(3) Exciccata of mushrooms from Japan;
(4) DNA barcoding from all mushroom species found in Tsukuba Botanical Garden.

6. Towards a strategy to enhance access to microbial resources

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Why are the huge investments in publicly funded research not protected for confirmation of results and future use? While documentation of gene and/or genome sequences in public databases and of type strains of novel prokaryotic species in at least two public service collections are two examples for a functioning implementation, deposition and release of material and data are, in practice, frequently left to the authors' discretion. Some journals may have a stricter implementation policy than others but generally enforcement mechanisms do not exist in those frequent cases where authors deny release of requested material by either not responding or by stating, among other arguments, intellectual property priorities, loss of material, biosecurity issues, export and import regulations, or patent issues. An alternative option to direct requests to authors for strains is the deposition of 'key' material in public collections. At a recent workshop participants agreed on a mechanism would ask authors during the submission process of manuscripts for an evaluation of the uniqueness of strains sufficiently novel to be worth depositing in public collections: These criteria could be:

- Uniqueness, based on a cutoff point of ≦98% of 16S rRNA gene sequence similarity to the most closely related species with a validated name.
- Metabolic uniqueness, based on the presence of a new pathway, modification of an existing pathway, metabolic differences compared to the type strain or novel products.
- Genomic uniqueness, such as significant differences (≧20%) in genome size, genome architecture or new regulatory mechanisms.
- Resources and parts thereof with fully sequenced genomes (microorganisms, phages, plasmids).
- A second strain of those species or subspecies for which only the type strain has been deposited.

The lecture will develop a strategy which would involve several stakeholders, such as editors, authors, collection manager as well as funding agencies for both research grants and public service collections.

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JSCC Symposia for the 60th Anniversary at IUMS2011 Sapporo

Session I. Phenotypic Diversity for Microbial Systematics
Organizer: Fred A. Rainey and Ken-ichiro Suzuki
Chairpersons: Fred A. Rainey and Hans-Jürgen Busse

1. Importance of chemotaxonomy for classification of bacteria

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Phylogenetic classification is a most important tool in bacterial taxonomy and usually the first hint that a new isolate belongs to a novel taxon. However, for proposal of a novel taxon its representative has to be characterized phenotypically and phenotypic traits have to be identified that are useful for differentiation from closely related taxa of the same level. In the case of proposal of novel species of established genera morphological, physiological and biochemical characteristics fulfill these requirements but due to heterogeneity among species of a certain genus these traits are often not suitable to characterize a genus. Chemotaxonomic characteristics such as fatty acids, polar lipids, quinones, peptidoglycan composition and polyamines are usually more conserved and, to some degree, quite well reflecting phylogenetic relationships. Hence, they are better applicable to describe genera and also to distinguish between genera. However, chemotaxonomic approaches have advantages and limitations and no generalization to which taxonomic level a certain approach is applicable for classification can be made. Fatty acid profiles are usually applied for species identification and may also contain genus-specific traits but in certain families they are not discriminative. Polar lipid profiles sometimes discriminate between species of a genus, often differentiating closely related genera or may be identical in representatives of different genera of a family. Peptidoglycan structure is often shared by all representatives of a genus but at least one example is known where strains of the same species differ in their peptidoglycan composition. Polyamine patterns may distinguish related genera, families or even higher taxa. The level, at which a certain approach is useful for classification, can be often identified by evaluation of the chemotaxonomic traits among phylogenetic relatives. In this contribution examples from fatty acid and polar lipid profiles, quinone system, peptidoglycan structure and polyamines are discussed based on data from literature.

2. Taxonomic implication of mycolic acid analysis by MALDI Spiral-TOFMS in suborder *Corynebacterineae*

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Mycolic acids are 2-alkyl 3-hydroxy fatty acids characteristic in the cell envelope of actinobacteria of the suborder *Corynebacterineae*. Mycolic acids are classified into subclasses based on the carbon chain length of the two alkyl chains and presence of double bonds and/or other functional bases in the carbon chain. Since mycolic acids are presumably related to both the physiology and the virulence of these bacteria, analysis of composition attracts attentions. To analyze them, GC-MS, HPLC, and electro spray ionization-MS (ESI-MS) have been mainly used[1)]. However, these techniques require high skill and tuning of the protocols in the pretreatments.

In this study, high resolution MALDI-TOFMS with a spiral ion trajectory (MALDI Spiral-TOFMS)[2)] was applied for mycolic acids. Mycolic acid samples were extracted from the cells of the genera *Rhodococcus*, *Gordonia* and *Dietzia*, and observed by MALDI Spiral-TOFMS without prior purification of individual subclasses. The high mass resolution of this equipment enables separation of adjacent peaks in mass spectra, which allows not only evaluation for the number of total carbons and double bonds, but also identification of each component. For both *Rhodococcus* and *Dietzia*, α-, α'- and saturated mycolic acids were mainly observed while saturated mycolic acids were absent for most of *Gordonia*, and ketomycolic acids were detected for *G. sputi*. The differences of amounts for each component were also evaluated among the sample strains. Rapid and easy analysis of mycolic acid composition is realized using MALDI Spiral-TOFMS, which enables analysis for a large

number of samples in short time. This method would be effective for the chemotaxonomic characterization of bacteria containing mycolic acids.

1) C. Barry et al., Prog. Lipid Res., 37, 143 (1998), 2) T. Satoh et al., J. Am. Soc. Mass Spectrom., 18, 1318 (2007).

3. Phenotypic variation among ecologically and genetically differentiated populations of *Saccharomyces cerevisiae*

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The budding yeast *Saccharomyces cerevisiae* is the most commonly used microbial agent for the production of bread, beer and wine and a powerful model system in genetics, genomics and molecular biology. However, little is known about its distribution and life histories in nature. Our recent field survey showed that *S. cerevisiae* commonly exists in highly diversified substrates from both man-made environments as well as habitats remote from human activity. The ubiquitously distributing *S. cerevisiae* occurs in surprisingly diverged clear populations. The oldest and other significantly differentiated basal lineages distribute in primeval forests; while close wild relatives of less diverged domestic lineages exist in man made environments. We have identified eight new wild and one new domestic *S. cerevisiae* lineages in China. We then investigated the stress tolerance of *S. cerevisiae* isolates from different lineages with different ecological origins, namely, nature, orchard, home-made sourdough starters and brewery. The orchard and sourdough starter groups were more robust than the other groups under the stressful conditions of ethanol and heat. The nature strains exhibited lower fitness under stressful conditions of ethanol and heat, but higher resistance to vanillin. Unexpectedly, the brewery group showed lower fitness than the orchard sourdough starter groups in the stressful conditions of ethanol and heat. These results indicate the correlations of stress tolerance with ecological and genetic differentiation in *S. cerevisiae* populations.

4. Phenotypic diversity of extremely halophilic Archaea and Bacteria and its exploitation in ecological studies of hypersaline environments

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Hypersaline lakes at or near salt saturation such as Great Salt Lake, the Dead Sea, and saltern crystallizer ponds are often inhabited by dense communities of extremely halophilic microorganisms: Archaea (members of the *Halobacteriaceae*) as well as Bacteria (*Salinibacter*). Phenotypic data collected during the polyphasic characterization of species of halophiles are useful not only in taxonomic frameworks, but can also be exploited in ecological studies to obtain information about the nature of the communities present and their activities in situ. Thin layer chromatography of polar lipids extracted from the community provides information about the genera of *Halobacteriaceae* present, and enabled the prediction of the type of glycolipid present in *Haloquadratum walsbyi* long before its isolation in pure culture. Mass spectrometry yields polar lipid patters at a higher resolution, and the detection of its unique sulfonolipids enables the assessment of the relative importance of *Salinibacter* in the community. HPLC analyses of pigments provide a quantitative estimation of the presence of *Halobacteriaceae* (C50 bacterioruberins) and *Salinibacter* (the C40-carotenoid acyl glycoside salinixanthin). *Halobacterium* is the only genus of *Halobacteriaceae* known to grow anaerobically on arginine, and this property was used for its selective isolation from natural environments. Some halophilic Archaea can use fumarate as electron acceptor, and this property can be demonstrated directly in the environment: fumarate addition lowers the oxygen consumption rate by saltern crystallizer communities. Differences in sensitivity of the diverse halophiles to antibiotics and other antibacterial compounds can be used to study the contribution of Archaea and Bacteria to the activities displayed by the communities. Thanks to their limited biodiversity and their often high prokaryote densities, hypersaline environments are thus ideal systems to study the phenotypic properties of the microbial community and to relate these to the polyphasic information available from taxonomic descrip-

tion of species isolated from such environments.

5. Phenotypic diversity of bacteria from arid environments

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Over 30% of the earth's land surface is classified as arid lands. Some of these arid lands fall into the hyper-arid category, receiving less than 25 mm annual precipitation. There are many areas in the Atacama Desert, Chile that are considered to be hyper-arid and in many years receive zero rainfall. Heterotrophic bacteria which have been cultured from these environments are present at very low levels and with limited diversity. Some soils from this hyper-arid region have been referred to as Mars-like due to their highly oxidizing nature and the extremely low levels of organic carbon that they contain. Isolation studies from both the surface and subsurface of these areas have provided a number of novel actinobacteria, deinococci and alphaproteobacteria. A large number of phenotypic characteristics have been studied for these isolates and their relatives that have been isolated from less arid environments. Considering the highly desiccated and highly oxidizing nature of the environments that these isolates inhabit, their resistance to desiccation, gamma and ultra-violet radiation, as well as their tolerance to sodium, magnesium and calcium perchlorates has been examined. Resistance to gamma radiation and desiccation has allowed us to selectively isolate addition taxa of these groups from soils collected from other arid environments. We have also examined these environmental related phenotypes in reference strains of the genera to which the novel isolates belong. Our data demonstrate that not all isolates from a single sample of arid soil share the same phenotype when it comes to tolerance of extreme conditions of desiccation, and exposure to gamma radiation and highly oxidizing compounds. We have also found that type strain of related taxa in culture collections have similar novel phenotypes that had not been examined. Such phenotypes constitute an important component of the taxonomic description of taxa isolated from arid environments.

Session II. Genomics and Resources in Microbiology

Organizer: Hans-Peter Klenk
Chairpersons: Kevin McCluskey

1. Characterization of classical mutant strains of *Neurospora* from the Fungal Genetics Stock Center Collection using gene and whole genome sequence analysis

Kevin McCluskey[1], Aric Wiest[1], Scott Baker[2]
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We have characterized 22 mutant strains of *Neurospora crassa* where the identity of the mutation is anonymous at the DNA sequence level. Our first efforts used chromosome walking and complementation of Temperature Sensitive mutants. This led to the characterization of four mutant strains which carried mutations originally mapped by Inoue and Ishikawa (1970). This work has led to the development of a novel selectable marker based on complementation of a temperature sensitive ribosomal protein gene. Eighteen additional strains have been characterized by whole genome sequencing. The genomes of eighteen classical mutants of *Neurospora crassa* were sequenced at the US Department of Energy Joint Genome Institute. The distribution of Single Nucleotide Polymoprhisms, insertions, and deletions, was analyzed with reference to genetic mapping data to identify mutations. The ability to compare among multiple strains simplified the analysis and allowed identification of putative mutations in most strains. The identification of hundreds of thousands of SNPs and indels allowed improvements to gene annotation and provided insight into the co-inheritance of large regions of the genome. The presence of significant numbers of mutations in these strains suggests that classical mutant strains carry a burden of neutral or unselected mutations. Additional features of the genome of *Neurospora*,

such as SNP distribution, background mutation rate, and indel size and selective distribution were evident in the dataset generated by whole genome sequencing of multiple strains.

2. Comparative genomics of *Aspergillus* and gene diversity

Masayuki Machida[1,2], Hideaki Koike[1], Yoshinori Koyama[1], Hiroko Hagiwara[1], Itaru Takeda[2], Myco Umemura[1], Koichi Tamano[1]

[1]Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Japan, [2]Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology

Various *Aspergillus* species have been sequenced to date. It was found that they shared 5,000-7,000 common genes and that the rests were mostly relating to metabolism. Secondary metabolites are low molecular weight compounds usually produced after active growth has ceased, many of which are bioactive. They are important for the interaction between fungi and plants, animals, microorganisms and other fungi. Recent accumulation of genomic information has uncovered a large diversity of fungal metabolic pathways for secondary metabolites. Interestingly they are highly enriched on the non-syntenic blocks. Phylogenetic and syntenic analyses suggested that secondary metabolism genes might be horizontally transferred. Possible source organisms, however, have not been found although more than 1,000 species have been sequenced.

We have found that genes on the sub-telomeric regions and the non-syntenic blocks have been more significantly mutated than those on the center part of a chromosome. It has been reported that the high mutation frequency might provide a mechanism to generate novel genes. We have also found that the genes on these regions are uniquely regulated differently from those on the other regions. Considering that secondary metabolism and secretory hydrolase genes are enriched on these regions, extensive comparative analyses between various fungi are thought to be extremely important for industrial applications and to understand how genes are generated and evolved.

3. The Genomic Standards Consortium: an open invitation to participate

George M Garrity

Microbiology & Molecular Genetics, Michigan State University and Editorial Office, Standards in Genomic Sciences, USA

With over 1500 genomes of Bacteria, Archaea and Eukarya completely sequenced and many thousands more in the pipeline in various stages of completion, is seems obvious that the life-sciences community has accepted this approach as the new gold-standard for describing and defining all that encompass life, as we know it. The universal applicability of the methods and the rapid decline in the cost of producing sequence data makes it impossible to argue against this approach. However, producing sequence data is the first and perhaps the least expensive step. Interpreting sequence data in a meaningful and consistent manner demands that it be annotated thoroughly and accurately so that it may be put into proper perspective and made available for reuse. This is not just a technical challenge, but a social challenge as well. The Genomic Standards Consortium (GSC) is an open-membership organization that has taken a leadership role in advocating for and promoting the development of community-based standards as they apply to all aspects of genomic, metagenomic and microbiome research. The goal of this presentation will be to provide a brief history of the GSC, describe ongoing activities and to invite members of the culture collection community to join us in our efforts to improve the quantity and quality of contextual information about genome, metagenome and marker gene sequences in public data repositories and knowledgebases.

4. The role of culture collections and DNA banks for large scale genomics

Hans-Peter Klenk
Microbiology, German Collection of Microorganisms and Cell Cultures, Germany

When the genomic era began about 15 years ago the impact on culture collections was barely noticeable, because many of the genomes sequenced at that time were neither type material nor were they deposited in culture collections. The situation changed only recently with large scale projects such as the Genomic Encyclopaedia of Bacteria and Archaea and the Human Microbiome Project, when larger sets of type strains were used for genome sequencing. Culture collections started to build DNA banks that serve as well as source for sequencing templates needed by the sequencing centres, as for scientist doing post-genomics follow-up lab work with strains that they often can barely grow in their labs.

Culture collections and their DNA banks do not only serve as a source of DNA for sequencing centres, but also as a source of meta- or contextual data linked to the strains. This is especially interesting when we consider that the knowledge about the phenotypic features of an organism is of significant importance for the functional interpretation of the genome sequences. This development in turn provides to collections also an enormous opportunity to use genome-based information as basis for systematic phenotyping of collection strains in order to improve and to optimize the growth conditions of their strains.

With currently only about 600 (7%) genome-sequenced type strains it is too early to seriously talk about an universal genome-based taxonomical system for the prokaryotes, but this is certainly an attractive and meanwhile feasible aim for microbial taxonomists. For the discrimination of type strains of species the Average Nucleotide Identity and other genome-to-genome comparison methods already allow a digital DNA-DNA-Hybridization, which is about to replace the classical labour intensive wet lab procedure requested for the formal description of novel archaeal and bacterial species names.

Session III. Culture Collection and Microbial Systematics

Organizer: Ken-ichiro Suzuki and Philippe Desmeth
Chairpersons: Takashi Ito and Philippe Desmeth

1. Microbial species descriptions: the importance of multiple strains

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Given the right cautionary preamble, most microbial systematists would probably agree that species can be defined as groups of individuals that share certain common characteristics and are distinct from other such groups. To establish groups as distinct requires an appreciation for the variance within the groups. It follows that species cannot be properly delineated if only a single representative of each species is available for study. The view presented here is that descriptions based on multiple isolates make for better science! If, for example, a sequence-based phylogenetic species concept is implied, as it often is, reciprocal monophyly must be established, which requires that the extent of sequence polymorphism be evaluated, at the very least in the new species. However, significant proportions of yeast species have been and continue to be described on the basis of a single isolate or a few isolates of dubious independence. This practice is even the object of vigorous advocacy (Kurtzman 2010). In recent years, the task of discovering new yeast species has been greatly enhanced by the availability of a continuously updated database of sequences for the D1/D2 domains of the large subunit rRNA gene. Kurtzman and Robnett (1998) have shown empirically that polymorphic species seldom exhibit more than three substitutions in that region and that well-defined species seldom differ by less than 1% substitutions. This observation has triggered a veritable avalanche of species descriptions based solely on this criterion, often applied to single strains. Recent evidence suggests that species that are sampled thoroughly may exhibit substantial amounts of polymorphism at the level of barcoding sequences. I shall review examples of such cases and discuss their potential impact on the problem of correct delineation and typification.

2. Use of MALDI-TOF MS as a phenotypic alternative to assess microbial species classification

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Matrix assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS), using 'intact' or whole cells has been shown to be an excellent tool for the identification of bacterial strains and should be applicable for initial classifications of large collections of isolated strains into phenotypic clusters for further, more detailed classifications or identifications. The great advantage of the method is that the analyses can be done using a minute amount of biomass from a given colony, without previous treatments or time-consuming extractions. The MS profiles, in part, reflecting the heterogeneity of cell ribosomal proteins, produce stable phenotypic characterisations.

We have studied large sets of isolates using MALDI-TOF MS, and contrasted the resulting clusters with genetic tools as 16S rRNA gene phylogenetic reconstructions, DNA-DNA hybridizations (DDH) and RAPD analysis. The study has been done by using new unknown isolates from environmental samples, as well as well studied isolates of the heterogeneous species Pseudomonas stutzeri. The results observed are very encouraging as MALDI-TOF MS clustering approach using whole cell profiles render units that can be identified as putative genomospecies with a certain degree of internal diversity. These preliminary analyses show that for circumscribing species, MALDI-TOF MS phenotypic profiles may be even of a better resolution than DDH, and thus an excellent complement when trying to classify new taxa.

If the use of MALDI-TOF MS for classification purposes is becoming a routine for taxonomists, a successful future is to foreseen as: (i) it generates cumulative and interactive profile databases, (ii) it produces cheap preliminary screening of large set of isolates with very accurate circumscriptions compatible with the current prokaryotic species circumscriptions, and (iii) if a profile of a new isolate matches with that of a known taxon, its identification is, with a very high chance, guaranteed.

3. Is species an essential unit of microbial systematics?

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Taxonomy has been circumscribed as the art of biological classification but is consisting of more than just classification (the orderly arrangements of recognizable units). In addition to classification, nomenclature, i.e. labelling of these units defined by classification and also identification of these units, are of central importance for a stable, practical and predictable system in all disciplines of microbiology. The ultimate goal of taxonomy is to establish a system that mirrors the order in nature. The term natural can be associated with different meanings, but is now most often associated with evolution. In prokaryote microbiology, the taxonomic concepts try to mirror the whole evolutionary order back to the origin of life with the cell as the basic unit showing all essential properties of life. Species are regarded as the fundamental units in taxonomy and microbial strains are most often assigned to the taxonomic ranks species and subspecies or at least to the genus level. With this nomenclatorial assignment (and simultaneously the assignment of the meaning behind this name), the link of a mental representation (the taxonomic rank) to a physical object is most obvious. Type strains serve in particular as reference points in the cases of the basic taxonomic units species and subspecies. And species are (automatically) assigned to a genus in a binomial combination according to the Linnean system. Many studies have been published on the species definition or species concepts in bacteriology and many different opinions have led to controversial discussions.

The criteria used for classifications may change in the future, when we have a full insight into the complexity of the genomes (and other omes) of microorganisms. However, the maintenance of stable and predictable taxonomic and nomenclatural standards (including the basic unit species) is even more important in these high-throughput times.

4. The roles of culture collections in microbial taxonomy

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Publicly accessible culture collections have increased the importance in the current microbial taxonomic practices. For examples, the culture collections have accepted deposits of type strains for proposal of novel bacterial and archaeal species/subspecies, and issue the certificates of deposits and availability to the depositors in accordance with the Bacteriological Code and the policy of IJSEM. Even after the publications and making the strains available, the collections are to check quality of them, on any occasions, and distribute these authentic materials to end-users. In addition, sharing such type strains among culture collections helps avoid loss of them. As a matter of course, the collections can perform these works based on the premise of the depositor's cooperation and responsibilities to the depositing strains. Here I would like to exemplify the current practice of the acceptance and quality management of the deposited microbial strains, particularly the type materials, at our culture collection, and underline the importance of the cooperation between culture collections and depositors/end-users, as well as among the culture collections.

5. Quality of culture collections to build microbiological research on firm ground World Federation for Culture Collections (WFCC), professionals underpinning microbial systematics

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Comprehensive exploration and structured study of the microbial diversity implies access to huge numbers of specimens of which some are referred to as "type strains" when they constitute the archetype of a species, and as "reference strains" when they are representative of a lineage with well identified properties. Type strains constitute the primary elements of taxonomy, reference strains are essential parts of coherent R & D processes. These assets of fundamental scientific importance must be conserved and provided with the highest level of reliability to ensure consistent research and knowledge build-up. Therefore the systematic deposit of studied microbial material in culture collections should be the rule.

Since the infancy of microbiology, scientists struggle to ensure long term ex situ conservation of living microbial material for further uses. This specialised work essential to build microbiological research on firm ground is performed by culture collections (CC). CCs are infrastructures specialised in long term conservation of microbial resources and management of related data and information. Their mission is to provide facilitated access to (technically and legally) fit-for-use microbiological resources of consistent quality with regard to the material itself as well as related data. These facilities are established all around the world and most of them are registered in the World Data Centre for Micro-organisms of WFCC (www.wfcc.info).

CC have evolved from mere centres of conservation and distribution of microbiological material to "Biological Resources Centres" (BRC) conceived as sources of all essentials for Research and Development in life sciences[1)]. To fulfil their role of basic infrastructure for biosciences in Knowledge Base Bio-Economy, they must implement quality management system for continuous improvement of their scientific expertise.

1) Biological Resource Centres Underpinning the future of Life Sciences and Biotechnology, 2001, OECD, Paris. http://www.oecd.org/dataoecd/26/19/31685725.pdf
OECD Best Practice Guidelines for BRC, 2007, OECD, Paris. http://www.oecd.org/dataoecd/6/27/38778261.pdf
WFCC Guidelines. http://www.wfcc.info/guidelines