

Abstracts - U.S. Culture Collection Network Workshop

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Overview of the ARS Culture Collection

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The Agricultural Research Service (ARS) Culture Collection in Peoria, IL, maintains more than 95,000 strains of agriculturally and industrially important bacteria and fungi. Most of these isolates are maintained in an open collection that distributes 6,000 – 8,000 strains annually in response to requests from scientists in the U.S. and around the world. The ARS Patent Culture Collection contains an additional 6,100 strains, many of which were deposited under the Budapest Treaty in association with patent applications in the United States and any of the 77 other countries that are contracting parties of this microbial patent deposition treaty. The ARS Culture Collection is directed by the Research Leader of the Bacterial Foodborne Pathogens and Mycology Unit and technical operation is handled by two support scientists and an administrative assistant. In addition, direct scientific expertise is provided by five Ph.D. microbiologists and/or geneticists who have authored more than 700 scientific publications. These scientists work with the ARS Office of National Programs to develop research and collection management objectives into a project plan, which undergoes peer review on a 5 year cycle. While the specific areas of research change over time, the major goals of this project are to enhance the quality, diversity, and utility of the ARS Culture Collection holdings, and to conduct and support microbiological research that advances agricultural production, food safety, public health, and economic development.

A Model Organism Collection Supported by the NSF, its Culture Collection and Data Systems

John Wertz, Director, E. coli Genetic Stock Center

The CGSC is a Genetic Stock Center as distinct from a Culture Collection. In short this means we have very few different taxa compared to a culture collection, but we know a great deal more about each culture in our collection. Tracking this information has been one of our primary challenges. I will show how data management has changed over the last 50 years at the CGSC including some of the pitfalls. There is a tradeoff between the benefits a standardized “one size fits all” information system and those of a custom “bespoke” data management system.

The impact of culture collections on molecular identification, taxonomy and solving real problems

David M. Geiser, Seogchan Kang, Todd J. Ward, Kerry O'Donnell, Vincent Robert and Pedro W. Crous

Among the fungi, *Fusarium* has stood out as a major focus for culture collection resource development over the last century. This has facilitated unprecedented molecular taxonomic advancements, which in turn has led to problem solving in plant pathology, mycotoxicology, medical mycology and basic research. One high impact advance has been the development and implementation of molecular identification resources. The first such tool developed was FUSARIUM-ID, a simple BLAST-enabled database that targeted a portion of the translation elongation factor 1-alpha gene for species-level identifications. Since then, FUSARIUM-ID has been expanded to include multilocus data from ~2000 *Fusarium* isolates, and a parallel platform (*Fusarium* MLST) has been implemented at the CBS-KNAW Biodiversity Centre in The Netherlands. A crucial benefit of these database tools is that all sequences are connected to vouchered, publicly available cultures, unlike GenBank, which has little or no control over accession information associated with sequence data or the availability of cultures. Future plans for *Fusarium* culture collections and associated cyber infrastructure include integration of genomic data and social networking tools. The epidemiological investigation of the 2005-06 outbreak of contact lens-associated fusarial eye infections will be presented as an example illustrating the impact of collections-enabled molecular systematics.

Leveraging culture collection assets with next-generation DNA sequencing at NCAUR.

Christopher A. Dunlap, Crop Bioprotection Research Unit, NCAUR, ARS, USDA, Peoria IL

The rapid improvements in high-throughput DNA sequencing have greatly improved the affordability of this technique to characterize microorganisms. The development of the NCAUR's next-generation DNA sequencing facility was implemented with the ability to leverage the culture collections assets as resources became available. The ability to sequence large numbers of microorganisms requires the development of processes to handle high-throughput culturing, DNA extraction, DNA fragmentation, NGS library preparation, sequencing and data analysis. This presentation will address the basics of high-throughput DNA sequencing and the capabilities at NCAUR's next-generation DNA sequencing facility. Then I'll address the approach and strategies we are utilizing to overcome the technical hurdles in converting microorganisms into genomes. The lessons learned from a 250 microbe pilot project will be discussed and how the required processes are being adapted with this new knowledge. The future role of high-throughput DNA sequencing on increasing the value of the culture collections will be explored.

The USDA Forest Fungi Collection

Ned B. Klopfenstein¹, Sara Ashiglar¹, John Hanna¹, Amy Ross-Davis¹, Geral McDonald¹ and Mee-Sook Kim²:

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Abstract: The Rocky Mountain Research Station (RMRS) has a unique collection of forest/tree-associated fungi with over 15,000 living specimens. Based in Moscow, ID, this USDA APHIS-PPQ (Plant Protection and Quarantine) containment facility houses fungal archives from approximately 35 states and 30 countries. The collection involves the work of at least 50 USDA Forest Service research scientists and collaborators over the past 30 years. Though the collection primarily focuses on species associated with Armillaria root disease from the Pacific Northwest it also contains other fungi, such as *Phellinus*, *Fusarium*, *Earliella*, *Microporus*, *Raffaelea*, tree-root endophytes, and fungi associated with wood decay. The RMRS Forest Pathology group and collaborating scientists continue to use and add to the collection for research in prediction of invasive pathogens, climate change, evolutionary relationships, and DNA-based identification of forest pathogens. Unfortunately, long-term maintenance of this invaluable collection is not funded and thus this collection is at risk of permanent loss. With an estimated 25 percent of specimens in the collection already dead, the RMRS Forest Pathology laboratory seeks a long-term solution to maintain the fungal archive collection.

Addgene

Joanne Kamens, Executive Director

Addgene is an independent, 501(c)3 nonprofit, bio repository located in Cambridge, Massachusetts. Addgene is dedicated to facilitating scientific discoveries by operating a plasmid library for researchers. Depositing to the library is free and there is a handling fee of \$65.00/plasmid for distribution. Requestors also cover shipping and there are reduced per plasmid handling fees for larger orders or for kits. Addgene has worked with over 1,500 laboratories to assemble its current collection of 23,000 plasmids, and continues to solicit new deposits. Addgene is able to distribute to any country and there are plasmids being sent to over 70 different countries. The current distribution rate is over 7,500 plasmids per month. By authenticating, storing, archiving, and distributing plasmids and their associated data, Addgene is creating a lasting resource for research and discovery scientists around the world.

Best Practices Guidelines and Other International Activities Relevant to Living Microbe Collections.

Kevin McCluskey, Fungal Genetics Stock Center, University of Missouri- Kansas City

Living microbe collections have become more widely recognized for their committed roles in protecting and preserving microbial diversity. The Organization for Economic Cooperation and Development published a "best practices" guideline for Culture Collections and this is similar in scope to the International Society for Biological and Environmental Repository (ISBER) Best Practices which have been updated multiple times. The OECD guidelines are directly targeted to living microbe collections and are written to accommodate activities in countries with different levels of economic development. At the same time, the Convention for Biological Diversity has imposed treaty limits on legal exchange of genetic material and these are being implemented through the Nagoya Protocol on Access and Benefit Sharing. The interests of living microbe collections are promoted regionally via a growing number of practical networks, and internationally by the World Federation for Culture Collections (WFCC). Recently the WFCC and related groups have promoted the idea of a Global Biological Resource Center Network which is made up of Nationally Certified Biological Resource Centers. Similarly, they are promoting a Biosecurity Code of Conduct for living microbe collections which emphasizes that collections are the best qualified and provide the safest way to conduct formal exchanges of living microbial materials. Ongoing efforts related to the Nagoya Protocol are developing methods to monitor international exchange of genetic material and the formal implementation of best practices for the legal and material aspects of such exchanges.

The National Plant Germplasm System: Current Status and Future Prospects

Peter K. Bretting, National Program Leader, USDA/ARS Office of National Programs, Beltsville MD

The USDA National Plant Germplasm System (NPGS) is one of the largest national plant genebank systems, conserving more than 560,000 samples from more than 14,800 species. The NPGS's 20 genebanks hold not only large collections of major staple crops, but also important collections of various horticultural and specialty crops. GRIN-Global, its information management system, is becoming an international standard for crop genebanks worldwide. The NPGS acquires, maintains, regenerates, documents, distributes, characterizes, evaluates, and enhances plant germplasm, and conducts research in support of the preceding activities. The demand for NPGS germplasm and associated information has grown substantially during recent years. During the last four years, the NPGS distributed more than 1 million samples to requestors worldwide. The research community also desires access to additional characterization and evaluation data to make more efficient use of the germplasm. Genomic research is generating thousands of new genetic stocks to be conserved. Furthermore, gaps in the collection must be filled, especially for crop wild relatives, which are not well-represented in the NPGS. The increasing demand for NPGS germplasm and increasing costs of labor, farming inputs, phytosanitary certificates, etc., contrast with the static or decreasing capacities to safeguard the expanding NPGS collections and meet the needs of the global crop research community.

The Larry F Grand Mycological Herbarium – From index cards to the worldwide web

Bryan R. Cody, Larry F. Grand and Marc A. Cubeta, North Carolina State University, Raleigh NC

The Larry F. Grand Mycological Herbarium (NCSLG) is housed in the Department of Plant Pathology at North Carolina State University. The collection was established in 1970 and consists primarily of wood decay and plant pathogenic fungi sampled from ecologically diverse habitats located in the southeastern US. In 2009, a National Science Foundation grant provided funds to improve the computerization and Internet presence of the collections. For the past five years, we have interacted closely with the Specify working group to develop a user-friendly and internet accessible electronic database to catalog our fungal specimens with related plant host and location information (www.cals.ncsu.edu/fungusherbarium).

Data from labels, field notes and annotations are manually keyed into customized forms in our Specify 6 software and the database is archived weekly. The database is on a server, therefore data entry and retrieval can be accomplished from client computers. Our approach for database development and management has provided a foundation to network with 26 mycological herbaria in the US as part of the recently funded NSF supported Digitization TCN Collaborative Macrofungi Collection Consortium project. For this project, selected data from our Specify database were uploaded and shared through the MycoPortal website (www.mycportal.org). The Specify group plans to have a web portal available July 2013 and it will allow us to share a fully searchable copy of our database on the web. Our database and website provide a valuable source of information for educators, scientists, and the general public on the biodiversity and distribution of fungi.

Data management and database structure at the ARS Culture Collection

David Labeda, Bacterial Foodborne Pathogens and Mycology Unit, USDA-ARS, Peoria IL

The organization and management of collection data for the 96,000 strains held in the ARS Culture Collection has been an ongoing process. Originally, the records for the four separate collections were maintained by individual curators in notebooks and/or card files and subsequently on the Center's mainframe computer system. A common set of database fields for microbial culture collections was developed during a meeting between the ARS GRIN database staff and the three ARS microbial culture collections held at Cornell University in the late 1980's to provide for future transportability and inter-operability. When our collection databases were migrated into FoxPro before the mainframe was decommissioned, this data structure was adopted for catalog relevant fields. The use of a unified set of field names, size and content for common catalog information across the 4 original databases facilitated the merging of the bacterial and actinobacterial holdings into a unified database during the last migration to the current PostgreSQL database system in 2001. Increased functionality has been added to the system, providing inventory management of both lyophilized and frozen materials and, most recently, an online order and request processing system. These additions have made the database schema far more complex than the original flat-file database. A new database has been developed to organize and provide public access to gene sequence data for multiple genes generated by studies characterizing collection materials and it is planned that this system will be linked to the collection database in the future.

The Role of NCGRP for Backup Security and Distribution of Microbial Collections

David Dierig, Josef Pohl, Vickie Hernandez, and Kim Webb

The USDA, ARS, National Center for Genetic Resources Preservation has supported the vision of establishing a national microbial collection by serving as the security backup repository for collections. Our current role is to increase the number of collections in backup storage and develop some technical knowledge on storage practices. We currently have 13 collections and a total of 10,600 isolates. The majority are being cryopreserved (LN₂ vapor) while a few are kept in conventional (-80 and -20°C) freezers. Three collections are being distributed through GRIN, although only a single valid request has been made so far. We will discuss the challenges of balancing information security with assuring the preservation of genetic diversity across the collections stored at the NCGRP. We will also present the ongoing developments to manage documents and workflow as well as the management of ex-situ microbe collections.

The ARS Culture Collection and Developments in Biotechnology

Cletus P. Kurtzman, Bacterial Foodborne Pathogens and Mycology Research Unit, NCAUR, ARS, USDA, Peoria IL

The ARS Culture Collection (NRRL) has played a prominent role in the development of biotechnology since its founding in 1940 when the Northern Regional Research Laboratory opened. Early discoveries included selection of production strains for penicillin, dextran blood extender, xanthan gum and the vitamin riboflavin. Later work included discovery of the first yeast to ferment D-xylose to ethanol, which gave impetus to production of liquid fuel from biomass. In recent years, extensive gene sequencing has led to the development of DNA sequence databases (barcodes) for essentially all known species of yeasts, the mold genera *Fusarium*, *Aspergillus* and *Penicillium*, and numerous bacteria including *Listeria*, *Bacillus* and the actinobacteria. These databases have markedly benefitted studies in food safety and biotechnology and are used by laboratories worldwide. In addition to rapid, accurate species identification, the DNA sequences can be used to develop phylogenetic trees, leading to the possibility of predicting genetic and metabolic properties for individual species. Examples to be presented included detection of sophorolipid surfactant production by members of the *Starmerella* yeast clade and modification of T-2 toxin from *Fusarium* by species of yeasts in the *Trichomonascus/Blastobotrys* clade.

The role of culture collections in national security

Alejandro P. Rooney, Ph.D., Crop Bioprotection Research Unit, NCAUR, ARS, USDA, Peoria IL

Within the broad spectrum of biodefense, surveillance and diagnostics efforts require sets of reference strains that “action agencies” can rely upon for accurate identification. In 2003, the ARS Culture Collection was approached by the U.S. Army for assistance in an investigation into an outbreak of severe pneumonitis during Operation Iraqi Freedom. Later that same year, the Federal Bureau of Investigation approached the ARS Culture Collection for assistance with the Amerithrax investigation. This presentation will cover the roles that the Collection played in helping those agencies with their respective tasks.

Culture collections support basic and applied plant disease research

Kerry O’Donnell, BFPM Research Unit, NCAUR-ARS-USDA, Peoria IL

The primary focus of my research is directed at understanding the genetic diversity, host range, and geographic distribution of *Fusarium*, one of the most important groups of mycotoxigenic plant pathogens. This research has benefited substantially from access to literally thousands of fusaria archived in U.S. (i.e., ARSEF, FRC and NRRL) and international culture collections (i.e., CABI-Biosciences, CBS-KNAW and DSMZ) over the past century and from collaborations with a global network of researchers working on the plethora of *Fusarium*-induced diseases of agronomically, horticulturally and silviculturally important crops. Strain typing using multilocus DNA sequence data has revealed that *Fusarium* is close to an order more species-rich than previously thought based on phenotypic data. Two studies facilitated by access to archived cultures include identification of the etiological agents responsible for *Fusarium* head blight of wheat and barley and those involved in the ambrosia beetle-*Fusarium* symbiosis. These studies provide plant disease specialists and quarantine officials with knowledge of pathogen distribution, diversity and mycotoxin potential crucial to minimize the threat of their inadvertent introduction into foreign areas and tools for their active molecular surveillance. The intimate integration of this research program with the ARS Culture Collection, FRC and CBS-KNAW helps ensure that the content and value of the germplasm these collections house and distribute will grow continuously to meet the ever increasing demands of agricultural biosecurity in a world interconnected by global trade.

Culture Collections and Public Health Research

Todd J. Ward, Bacterial Foodborne Pathogens and Mycology Unit, USDA-ARS, Peoria IL

Listeria monocytogenes is a foodborne pathogen capable of causing serious invasive illness in humans and other animals, and a leading cause of food recalls. The establishment and growth of a research collection of *Listeria* isolates has been critical in developing an evolutionary genetic framework, novel subtyping tools, and information on subtype-specific niche adaptation required to advance understanding of *Listeria monocytogenes* biology and to improve outbreak detection, source tracking, epidemiological investigation, and risk-based monitoring of this important foodborne pathogen. The ARS Culture Collection maintains nearly 2,000 strains of *Listeria monocytogenes* representing the genetic, phenotypic, and ecological diversity of this species. The molecular subtyping tools and data on *L. monocytogenes* diversity developed by scientists at the ARS Culture Collection have been used to support CDC, USDA-FSIS, and other public health agencies in outbreak investigations and in the development of risk-based sampling programs designed to reduce the public health and economic burdens imposed by this pathogen. Large and diverse collections of microbial cultures and associated metadata are critical to the growing number of large-scale genome sequencing projects. Contributions of the ARS Culture Collection to the 100K Pathogen Genome Project and the establishment of a reference database of whole genome sequences for use in outbreak investigations and source-tracking efforts will be highlighted.

Long term sustainability of NSF and other living collections

Mark A. Largent, Ph.D., Associate Dean, Lyman Briggs College/Associate Professor, James Madison College, MSU

As a historian of science and the AAAS Science & Technology Policy Fellow at the National Science Foundation in 2012-13, I was asked to analyze the history of NSF's support for living stocks collections over the last 60 years. In August 2012 the NSF invited representatives from about two dozen collections and agencies to a meeting that would help them understand the history of funding for living stocks and its prospects for the future. Our analysis demonstrated that, even though funding for living stocks collections have increased substantially (about 9-fold between 1985 and 2000), the number of funded proposals dropped in half. The increasing costs associated with such collections, their usefulness, and their relationships to other funding priorities are idiosyncratic and complicated. Nonetheless, the foundation fought to maintain funding. Recent changes that NSF has been compelled to make will demand a substantial consideration by the community of how best to continue to benefit from the foundation's support. Other collections - both scientific and non-scientific in nature - offer ideas for how the living stocks community can move forward.

Redesigning the Microbial Research Commons

Paul F. Uhler, National Research Council, Washington DC

Culture collections have operated in the public domain for many years as a resource to be shared internationally for both upstream research and downstream applications. This system has been increasingly under attack over the past two decades, however, and there are strong pressures to make it more proprietary and less accessible and usable. These pressures arise from many sources, whether legal, economic, political, or scientific. This presentation will briefly review these challenges and discuss several ideas for a redesigned commons for microbial cultures, and related digital data and information. The presentation summarizes some findings from a book on this topic, which I have been writing with two co-authors, Jerome H. Reichman of Duke Law School and Tom Dedeurwaerdere of the Universite Catholique de Louvain.