AEM Journals.ASM.org

The United States Culture Collection Network (USCCN): Enhancing Microbial Genomics Research through Living Microbe Culture Collections

Kyria Boundy-Mills,[a] Matthias Hess,[b,c,d] A. Rick Bennett,[e] Matthew Ryan,[f] Seogchan Kang,[g] David Nobles,[h] Jonathan A. Eisen,[i] Patrik Inderbitzin,[j] Irnayuli R. Sitepu,[a] Tamas Torok,[k] Daniel R. Brown,[l] Juliana Cho,[m] John E. Wertz,[n] Supratim Mukherjee,[c] Sherry L. Cady,[o] Kevin McCluskey[p]

Phaff Yeast Culture Collection, Food Science and Technology, University of California, Davis, Davis, California, USA[a]; Department of Animal Science, University of California, Davis, Davis, California, USA[b]; DOE Joint Genome Institute, Walnut Creek, California, USA[c]; Chemical Biological Process Development Group, Pacific Northwest National Laboratory, Richland, Washington, USA[d]; Plant Pathology, University of Arkansas, Fayetteville, Arkansas, USA[e]; The Genetic Resources Collection, CABI, Surrey, United Kingdom[f]; Department of Plant Pathology & Environmental Microbiology, Pennsylvania State University, University Park, Pennsylvania, USA[g]; The Culture Collection of Algae, The University of Texas at Austin, Austin, Texas, USA[h]; Genome Center, University of California, Davis, Davis, California, USA[i]; Plant Pathology, University of California, Davis, Davis, California, USA[j]; Lawrence Berkeley National Laboratory, Berkeley, California, USA[k]; Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA[l]; Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, California, USA[m]; Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut, USA[n]; The Culture Collection of Microorganisms from Extreme Environments, Pacific Northwest National Laboratory, Richland, Washington, USA[o]; Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA[p]

The mission of the United States Culture Collection Network (USCCN; http://usccn.org) is "to facilitate the safe and responsible utilization of microbial resources for research, education, industry, medicine, and agriculture for the betterment of human kind." Microbial culture collections are a key component of life science research, biotechnology, and emerging global biobased economies. Representatives and users of several microbial culture collections from the United States and Europe gathered at the University of California, Davis, to discuss how collections of microorganisms can better serve users and stakeholders and to showcase existing resources available in public culture collections.

Culture collections are essential resources for preserving biodiversity, and coordination among collections is critical to ensure their long-term viability and the success of the U.S. scientific enterprise. The United States Culture Collection Network (USCCN) is a new organization that has emerged from a National Science Foundation Research Coordination Network (grant no. DBI1203112). This network allows users to share best practices and develop collaborations and promotes interactions among U.S. and international culture collection curators. By the start of its third year, the USCCN had hosted five formal events at participating U.S. collections. For many participants, these meetings represent the first time they have interacted with or even visited a collection other than their own.

The most recent USCCN meeting, held at the University of California, Davis (UC Davis), 24 to 25 September 2014, engaged the genome and metagenome research communities and included collection curators from a dozen U.S. collections and one European collection, along with a diverse set of collection users and stakeholders from academia, government, and industry. The meeting participants discussed ways to improve interactions between collections and genome researchers to advance the common goal of furthering education, research, and technological development using the collections. Collection curators gave oral or poster presentations to showcase the history, holdings, uses, and availability of materials from their collections. A list of the participating collections is presented in Table 1. Select presentations from the meeting can be viewed online at http://f1000.com/posters/browse/summary/1097073.

Recurring themes during the meeting included preservation of biodiversity, promotion of reproducibility, access to both strains and associated data, robust taxonomy, quality control, pricing structure, stock preservation, succession, long-term planning, visibility, and broadening services, some of which are summarized below.

Genome sequencing and biodiversity. Culture collections play a crucial role by providing correctly identified strains and associated metadata for DNA sequencing and by accessioning strains from sequencing projects. Three massive microbial-genome sequencing projects are filling taxonomic gaps and expanding knowledge in critical areas, such as pathogenicity, carbon cycling, and bioenergy. These are the Genomic Encyclopedia of Bacteria and Archaea (GEBA) (http://jgi.doe.gov/our-science/science-programs/microbial-genomics/phylogenetic-diversity/), the 100K Food-borne Pathogen Genomes Project (100KFPG) (http://100kgenome.vetmed.ucdavis.edu), and the 1000 Fungal Genomes Project (1KFG; http://genome.jgi.doe.gov/programs/fungi/1000fungalgenomes.jsf).

GEBA is a collaboration between the U.S. Department of Energy (DOE) Joint Genome Institute and the Deutsche Sammlung

Accepted manuscript posted online 19 June 2015

Citation Boundy-Mills K, Hess M, Bennett AR, Ryan M, Kang S, Nobles D, Eisen JA, Inderbitzin P, Sitepu IR, Torok T, Brown DR, Cho J, Wertz JE, Mukherjee S, Cady SL, McCluskey K. 2015. The United States Culture Collection Network (USCCN): enhancing microbial genomics research through living microbe culture collections. Appl Environ Microbiol 81:5671–5674. doi:10.1128/AEM.01176-15.

Editor: D. Cullen

Address correspondence to Kevin McCluskey, mccluskeyk@ksu.edu.

This article is contribution no. 15-399-J from the Kansas Agricultural Experiment Station.

doi:10.1128/AEM.01176-15

TABLE 1 Participating collections at the fall 2014 USCCN meeting

Collection name and acronym	Collection Web address
National Center for Marine Algae (NCMA)	https://ncma.bigelow.org
The Culture Collection of Algae (UTEX)	http://www.utex.org
The Culture Collection of Microorganisms from Extreme Environments (CCMEE)	http://uoregon.technologypublisher.com/technology/2486
The Mollicutes Collection (TMC)	http://www.IOM-Online.org
The Coli Genetic Stock Center (CGSC)	http://cgsc.biology.yale.edu
University of California Davis, Wine Microbe Collection (UCDVEN)	http://wineserver.ucdavis.edu/industry/enology/culture/index.html
University of California Davis, Phaff Yeast Culture Collection (UCDFST)	http://phaffcollection.ucdavis.edu
Fungal Genetic Stock Center (FGSC)	http://www.fgsc.net
Fusarium Research Center (FRC)	http://www.fusariumdb.org/index.php
Department of Defense Unified Culture Collection	http://www.usamriid.army.mil/ucc/index.cfm
Critical Reagents Program	http://www.beiresources.org/About/CriticalReagentsProgram.aspx
USDA Forest Service, Center for Forest Mycology Research	http://www.fpl.fs.fed.us/research/centers/mycology/culture-collection.shtml
University of California at Berkeley, Microgarden Algal and Fungal Teaching Collection	http://www.microgarden.berkeley.edu/microgarden
CABI, United Kingdom, Genetic Resources Collection	http://www.cabi.org/services/microbial-services/culture-collection/

von Mikroorganismen und Zellkulturen (DSMZ) in Braunschweig, Germany. GEBA highlights that living microbe collections are a source of high-quality DNA from vouchered and publicly available microbial resources for benchmark genome projects (1). GEBA has spawned several offshoot projects, including the One Thousand Microbial Genomes (KMG-I) project (2), which is a systematic effort to fill in the evolutionary gaps in the prokaryotic branches of the tree of life by sequencing 1,000 phylogenetically diverse type strains. A more focused project, the 100K Foodborne Pathogen Genomes Program at UC Davis, seeks to improve food safety by cataloging pathogens from food and the environment and includes a new collection as part of its mission plan. Finally, the 1KFG project will provide genome sequence data from every fungal family. The organizers of GEBA and the 1KFG project have explicitly emphasized the value of linking whole-genome sequencing projects with established culture collections. Hence, they acknowledge that taxonomic type strains may not be immediately available or even desirable as whole-genome reference strains (3). Related projects utilized large numbers of native or modified microbial strains for discovery of novel products, such as biofuels (4), for analysis of stress tolerance (5, 6) and of metabolites and pigments, and for the characterization of anonymous mutated genes from the classical genetics era (7). Many of these discoveries were made decades after the relevant microbes were originally isolated and preserved. They may have been isolated for uses that the original collector or depositor did not envision at the time the sample was deposited. Together, these factors emphasize the importance of preserving microbes for future uses that we cannot anticipate today.

Just as taxonomic codes such as the International Code of Nomenclature of Bacteria require that type strains of new species must be deposited in public repositories and distributed without restrictions, microbes used in genome sequence publications should also be deposited and made available to the scientific research community, perhaps as a requirement for publication or funding. Some journals, including those of the American Society for Microbiology (ASM), already advocate the deposition of microbes cited in publications into publicly accessible culture collections. As evidence of the engagement of the USCCN, a press release was issued in support of the ASM's September 2014 statement urging researchers to perform an inventory and deposit microbes into appropriately managed public repositories (see http://www.digitaljournal.com/pr/2167058 for more information).

Extended data. The Global Catalog of Microorganisms (GCM; http://gcm.wfcc.info) is an aggregate catalog of over 60 global microbial collections, including several U.S. collections (8). This is an important step in ensuring that data associated with microbial strains, such as the geographic source, habitat of origin, and phenotypic properties, are publicly available. Many collections already have extensive in-house data but need support to develop their own online databases or to organize data for inclusion in GCM and also for submission to Straininfo.net, the Global Biodiversity Information Facility, and other data clearinghouses.

Community of collections. Different types of collection facilities have participated in USCCN activities in recent years. Among these are genetic stock centers which contain large numbers of closely related genetic variants, as well as biodiversity collections which preserve natural biodiversity across different taxon levels (Table 1). Recent reviews have explored the diversity of culture collections in the United States and globally (9–14). The mission statements of these very different culture collections have several goals in common, including the following:

- Obtaining microbes with scientific, ecological, commercial, or other value.
- Validating their taxonomic, genetic, phenotypic, morphological, or other properties.
- Preserving microbes under appropriate conditions to maintain them for future generations (15).
- Distributing strains to researchers in compliance with all applicable regulations and treaties.

To meet these needs, collections must include or have access to expertise spanning taxonomy, microscopy, cultivation, preservation, quality control, biosafety, regulatory compliance, financial management, information management, public relations, and customer service (Table 2). Concerted governmental and institutional support for training of curators and maintaining expertise

TABLE 2 Links to additional information

Agency or organization	Internet address
The United States Culture Collection Network	http://www.usccn.org
USDA Animal and Plant Health Inspection Service (APHIS)	http://www.aphis.usda.gov/wps/portal/aphis/ourfocus/importexport
International Air Transport Association (IATA)	http://www.iata.org/publications/dgr/Pages/index.aspx
Convention on Biological Diversity (CBD)	http://www.cbd.int
CBD Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization (ABS)	http://www.cbd.int/abs/about/
Organization for Economic Co-operation and Development (OECD) Best Practices Guidelines for Biological Resource Centres	http://www.oecd.org/sti/biotech/38777417.pdf
Uniform Biological Material Transfer Agreement	http://grants.nih.gov/grants/guide/notice-files/not94-204.html
World Federation for Culture Collections	http://www.wfcc.info/guidelines

in these diverse areas is essential. Group efforts for developing legal frameworks, databases, and quality control standards and protocols for culture collections are being developed in other parts of the world. These include the European Strategy Forum on Research Infrastructures' Microbial Resource Research Infrastructure (MIRRI; http://www.mirri.org/home.html) and the Asian Consortium for the Conservation and Sustainable Use of Microbial Resources (http://www.acm-mrc.asia/). These programs, supported by considerable governmental funding, have stimulated productive discussions among regional nodes and have led to significant improvements in the status of microbial biological resource centers in other countries (16). MIRRI, for example, is achieving its goals by aiming to connect resource holders with researchers and policy makers, through the establishment of a pan-European distributed research infrastructure that provides improved access to high-quality microbiological resources, services, and facilities for research, innovation, development, and application within a defined legal framework. (http://www.mirri.org/home.html). MIRRI interacts with the European Culture Collections' Organization (ECCO), which currently involves more than 60 culture collections in 24 European countries holding more than 400,000 deposits. Established in 1982, ECCO has been the incubator of several major European Union-funded global networking and research projects, including the Micro-Organisms Sustainable Use and Access Regulation International Code of Conduct (http://bccm.belspo.be/projects/mosaicc) and the Global Biological Resource Centre Network (http://www.gbrcn.org/) demonstration project.

Quality control and certifications. Quality control testing of microbes used in the genome sequencing projects presented at this meeting revealed that a small but considerable fraction of strains were misidentified or contaminated. Validating and tracking the purity, identity, and provenance of microbial strains are among the most important functions of a culture collection and are required for a collection to be considered a Biological Resource Centre (BRC) according to the guidelines published by the Organization for Economic Cooperation and Development (17). Moreover, to be considered a BRC collection, certifications and external validation are necessary. Several standards are available for collections, including those from the International Organization for Standardization (ISO). The best-known quality management standard is ISO 9001, which is a management standard. The most recent version, ISO 9001:2008, provides guidance and tools for organizations to ensure that they consistently meet customer requirements. For culture collections, this standard primarily impacts customer service, record keeping, and implementation of appropriate best practices. ISO 34:2009 deals with the provision of reference material, with the emphasis on recognizing the competence of the provider. The only standard that was specifically written for culture collections is the French standard NF S 96-900, which deals with every aspect of living-microbe and tissue biobanks. Culture collections must operate within international standards. Although the United States did not ratify the Convention on Biological Diversity (CBD), U.S. culture collections do exchange microbes with researchers in other countries. The international TRansparent User-friendly System of Transfer for Science & Technology (TRUST; http://bccm.belspo.be/projects/trust) initiative specifies that culture collections are intermediaries between the depositors and do not claim ownership of the material. The culture collections' responsibilities therefore include informing clients of the source and date of strain isolation, enabling researchers to determine whether they are obligated to share the benefits arising from commercialization.

Future prospects. Many culture collections represented at this meeting reported a time in their history when the collection was almost lost because the sole curator retired or died. A prospective succession plan is therefore crucial and may include support for moving a collection to another institution. The NSF Collections in Support of Biological Research program funds relocation of collections on a competitive basis, and the USCCN is to hold a meeting in late 2015 at the U.S. Department of Agriculture National Center for Genetic Resources Preservation focusing on endangered and orphaned collections. Moreover, the America COMPETES Reauthorization Act of 2010 (section 104) requires that the U.S. Office of Science and Technology Policy develop policies for management of scientific collections and that a review be conducted of the value of the collection prior to disposal of the collection. That review is mandated to include stakeholders from the research community (18). To capitalize on the inherent value of collections, a variety of additional services and materials providing diverse revenue streams for many collections around the world (19) were showcased. The staff members of some collections design, assemble, and distribute kits for the education of children in kindergarten throughout grade 12 (K–12). Similarly, curators of collections were encouraged to consider preserving mixed consortia in addition to pure microbial strains (20). Finally, the practice of banking and distributing genomic DNA, rather than the live organisms, removes the need to cultivate and preserve the organism. Some collections have responded to this request already; many collections distribute genomic DNA, and the Culture Collection of Algae at the University of Texas at Austin (UTEX) and National Center for Marine Algae and Microbiota

(NCMA) sell products derived from algal strains. The Coli Genetic Stock Center (CGSC) offers DNA products as well as genetic modification of *Escherichia coli* strains as a service. Another method of generating revenue is to provide space for visiting scientists (19). For example, the UTEX operates a pilot plant that enables large-scale cultivation of algae (http://web.biosci.utexas.edu/utex/algae-growth-facility.aspx).

Summary. Most universities have biological collections, and an effort has always been made at USCCN activities to engage the host community to identify and showcase the diversity of biological collections. The meeting at UC Davis included visits to the Bohart Museum of Entomology (http://bohart.ucdavis.edu/) and the Museum of Wildlife and Fish Biology (http://mwfb.ucdavis.edu/). These interactions reinforced the importance of promoting collections of every type. The formation of the USCCN, through the efforts of a consortium of collection scientists and collection users, is protecting and preserving valuable biological resources in the absence of new funding sources. Collection scientists are mobilizing intellectual resources to plan strategically and frame an initiative such as a national culture collection system to better protect and preserve valuable resources. USCCN meetings have generated knowledge and relationships that will help culture collections to better support users by archiving and disseminating key materials and services, emphasizing the value of reproducible taxonomy, and expanding user capacity for discovery. USCCN meetings have built bridges among curators of diverse types of collections and between collection curators and users. Participants learned of materials, services, and expertise available at several U.S. and international culture collections, as well as of the limitations and challenges that many culture collections face. Implementation of additional workshops and exchanges by the USCCN will engage scientists and researchers at collection facilities who are responsible for microbial collections of all types and sizes. Participation is always welcome, and interested parties are invited to contact the USCCN steering committee or register their interest at the USCCN website (http://www.usccn.org/contact/Pages/default.aspx). Future meetings will help curators to improve microbial products and services to meet the increasingly complex needs of 21st century research and may help funding agencies target support to the most critical activities.

ACKNOWLEDGMENTS

The Research Coordination Network (RCN) for a community of *ex situ* microbial germplasm repositories is supported by grant 1203112 from the U.S. National Science Foundation Division of Biological Infrastructure. Boundy-Mills and the Phaff Yeast Culture Collection are partially supported by grant 1349395 from the U.S. National Science Foundation Division of Biological Infrastructure. The Fungal Genetics Stock Center was supported by grant DBI 0235887 from the U.S. National Science Foundation. S.L.C. and the Collection of Microorganisms from Extreme Environments (CCMEE) are supported by the Environmental Molecular Sciences Laboratory (EMSL), a DOE Office of Science User Facility sponsored by the Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory (PNNL).

The American Phytopathological Society provides administrative support to the USCCN effort. The Department of Viticulture and Enology at the University of California, Davis, provided a meeting venue.

REFERENCES

1. **Wu D, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova NN, Kunin V, Goodwin L, Wu M, Tindall BJ.** 2009. A phylogeny-driven genomic encyclopaedia of Bacteria and Archaea. Nature **462:**1056–1060. http://dx.doi.org/10.1038/nature08656.
2. **Kyrpides NC, Woyke T, Eisen JA, Garrity G, Lilburn TG, Beck BJ, Whitman WB, Hugenholtz P, Klenk H-P.** 17 December 2013, posting date. Genomic encyclopedia of type strains, phase I: the one thousand microbial genomes (KMG-I) project. Stand Genomic Sci http://dx.doi.org/10.4056/sigs.5068949.
3. **Federhen S.** 14 December 2014, posting date. Type material in the NCBI Taxonomy Database. Nucleic Acids Res http://dx.doi.org/10.1093/nar/gku1127.
4. **Sitepu I, Jin M, Fernandez J, Sousa L, Balan V, Boundy-Mills K.** 2014. Identification of oleaginous yeast strains able to accumulate high intracellular lipids when cultivated in alkaline pretreated corn stover. Appl Microbiol Biotechnol **98:**7645–7657. http://dx.doi.org/10.1007/s00253-014-5944-8.
5. **Sitepu I, Selby T, Zhu S, Lin T, Boundy-Mills K.** 2014. Carbon source utilization and inhibitor tolerance of 45 oleaginous yeast species. J Ind Microbiol Biotechnol **41:**1061–1070. http://dx.doi.org/10.1007/s10295-014-1447-y.
6. **Sitepu I, Shi S, Simmons BA, Singer S, Boundy-Mills K, Simmons C.** 2014. Yeast tolerance to the ionic liquid 1-ethyl-3-methylimidazolium acetate. FEMS Yeast Res **14:**1286–1294. http://dx.doi.org/10.1111/1567-1364.12224.
7. **McCluskey K, Wiest A, Grigoriev IV, Lipzen A, Martin J, Schackwitz W, Baker SE.** 2011. Rediscovery by whole genome sequencing: classical mutations and genome polymorphisms in Neurospora crassa. G3 (Bethesda) **1:**303–316. http://dx.doi.org/10.1534/g3.111.000307.
8. **Ma J, Sugawara H.** 2011. Activities of World Federation for Culture Collections (WFCC) World Data Centre for Microorganisms (WDCM). Microbiol Cult Coll **27:**79–81.
9. **McCluskey K, Wiest A, Boundy-Mills K.** 2014. Genome data drives change at culture collections, p 81–96. *In* Nowrousian M, Esser K (ed), The Mycota, vol XIII. Fungal genomics, 2nd ed. Springer-Verlag, Berlin, Germany.
10. **Stromberg PM, Dedeurwaerdere T, Pascual U.** 2013. The heterogeneity of public ex situ collections of microorganisms: empirical evidence about conservation practices, industry spillovers and public goods. Environ Sci Policy **33:**19–27. http://dx.doi.org/10.1016/j.envsci.2013.04.003.
11. **Smith D, Fritze D, Stackebrandt E.** 2013. Public service collections and biological resource centres of microorganisms, p 267–304. *In* Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (ed), The prokaryotes: prokaryotic biology and symbiotic associations, 4th ed. Springer-Verlag, Berlin, Germany. http://dx.doi.org/10.1007/978-3-642-30194-0_14.
12. **Parsons JP, Duke CS.** 2013. Strategies for developing and innovating living stocks collections: an ESA workshop report. Bull Ecol Soc Am **94:**118–129. http://dx.doi.org/10.1890/0012-9623-94.1.118.
13. **Smith D.** 2012. Culture collections. Adv Appl Microbiol **79:**73–118. http://dx.doi.org/10.1016/B978-0-12-394318-7.00004-8.
14. **Uhlir PF, Simione F.** 2011. American Type Culture Collection: a model for biological materials resource management, p 63–68. *In* Uhlir PF (ed), Designing the microbial research commons: proceedings of an international symposium. National Academies Press, Washington, DC.
15. **Stacey GN, Day JG.** 2014. Putting cells to sleep for future science. Nat Biotechnol **32:**320–322. http://dx.doi.org/10.1038/nbt.2869.
16. **Smith D, Fritze D, Stackebrandt E.** 2013. Public service collections and biological resource centers of microorganisms, p 267–304. *In* Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (ed), The prokaryotes. Springer-Verlag, Berlin, Germany.
17. **Organization for Economic Co-operation and Development (OECD).** 2007. OECD best practice guidelines for biological resource centres. Organization for Economic Co-operation and Development, Paris, France.
18. **America COMPETES Reauthorization Act of 2010.** 2010. Public law 111-358. http://www.gpo.gov/fdsys/pkg/PLAW-111publ358/pdf/PLAW-111publ358.pdf.
19. **Smith D, McCluskey K, Stackebrandt E.** 2014. Investment into the future of microbial resources: culture collection funding models and BRC business plans for biological resource centres. Springerplus **3:**81. http://dx.doi.org/10.1186/2193-1801-3-81.
20. **Cary S, Fierer N.** 3 November 2014, posting date. The importance of sample archiving in microbial ecology. Nat Rev Microbiol http://dx.doi.org/10.1038/nrmicro3382.