

## Microbial Genetic Resources in the USA

USDA National Genetic Resources Advisory Council

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### Executive summary

*Microbial collections preserve, characterize, and distribute validated strains of bacteria, archaea, viruses, yeasts, and filamentous fungi for agriculture, industry, health, and research.*

As a global leader in agricultural productivity, the United States depends on access to validated microbial strains for diagnostics, plant and animal growth promotion, reduction of toxins on plant products, biological pest control, and for post-production processing and industrial productivity

- Diagnosis is the identification of microbes. It requires validated reference material to allow identification to genus and species, as well as pathogenic race (virulence against specific plant cultivars), toxin-producing potential, and mating type.
- Microbes for plant growth promotion include nitrogen-fixing bacteria, root symbiotic mycorrhizal fungi, and a growing list of endophytic microbes that can protect plants from subsequent infection, allow plants to grow at elevated temperature, or grow in hyper-saline environments.
- Microbes are essential for nutrient cycling in natural and agricultural soils.
- Pretreatment of certain crop plants with non-toxin producing microbes is used to prevent later colonization by toxin forming microbes.
- Biocontrol is use of living organisms to eliminate pests. In the present context it refers to the introduction of plant or pest-pathogenic microbes with a specific, narrow host range.
- Microbes are used extensively as cell-factories to produce enzymes, value-added chemicals, fermented foods, and diverse products of biotechnology.
- Research into plant protection, animal husbandry, and plant microbe interactions depends upon access to validated microbes.

The US Department of Agriculture, Agricultural Research Service (USDA ARS) has several important living microbe collections. Additional agriculturally relevant collections are supported by the US National Science Foundation (NSF), by US universities, private companies, or are self-supporting. Collections supported by the National Institutes of Health, the Department of Homeland Security, or the Centers for Disease Control are outside the scope of the current document.

While research publications are important, the microbes used in agricultural research are valuable and often represent the real output of that work. There is no public program for the preservation, cataloging and databasing, and distribution of these research outputs. This gap has created a situation where work may be unnecessarily repeated because the original material is not available.

To ensure that US agriculture, industry, and research are on the soundest foundation and have the resources to continue world-leading productivity and research, a systematic program of managing microbial resources is essential. Because the present system of microbial resource maintenance is distributed across multiple agencies, and because some microbial resources have passed into private hands, this system should have a broad mandate to manage public resources that are important to agriculture.

### **Background**

Microbial genetic resources support diverse aspects of agriculture and human well-being. Beginning in paleolithic times, the domestication of microbes contributed to production of fermented beverages, bread, and preserved foods. While the nature of the organisms involved was unknown, even the Roman Pantheon included Robigus, a lesser god of wheat stem rust, and the Bible mentions blighting and blasting of plants in several passages.

The impact of plant pathogenic microbes on agriculture continues into the present day and accounts for losses of over \$20 Billion annually, despite the extensive employment of crop protection measures like fungicides, antibiotics, cultural practices, and genetically resistant plants. Further, mycotoxins produced by pathogenic and post-harvest fungi can reduce the value of many crops and endanger human health. However, not all microbes affect crop production negatively.

Biocontrol is the practice of using beneficial organisms to limit the impact of pests. This can include the application of bacteria, such as *Bacillus*, or of arthropods that prey upon other insect pests. Bacterial biocontrol organisms can target pathogenic fungi, parasitic plants, or arthropods. For most of these, a single release is not sufficient to control the target pest. In some instances, a biocontrol organism can become established in the ecosystem, providing long-term control of a pest.

Beneficial microbes include root-nodulating bacteria, mycorrhizal fungi, and other microbes on the surface or interior of plants that influence subsequent growth characteristics. Among these, non-toxicogenic fungi can be applied directly to plants, seeds, or to soil to pre-colonize plants and prevent subsequent colonization by mycotoxin producers. This has benefits to large and small farmholders and to animal production directly. Both human and turkeys (at least) are highly vulnerable to aflatoxin toxicity.

Similarly, diverse products are available to change the plant microbiome (the Phytobiome) such that beneficial microbes out-compete pathogens and provide positive growth-promoting characteristics. Among the most desirable characteristics, tolerance of abiotic stresses like drought and salinity as well as pathogen resistance can be conferred by microbes that live inside the plant. New genome sequencing and culturing techniques are providing insight into the composition of these microbial consortia.

By providing characterized reference materials, collections of microbes serve taxonomic and diagnostic needs. To name an organism the Botanic Code of Nomenclature requires that every identified species must be represented by viable and preserved specimens. In the US, the American Type Culture Collection has the role of preserving living reference material (“Type strains”) for taxonomic purposes. Other formal collections such as the USDA ARS NRRL collection also support this requirement.

Accurate diagnosis of plant and animal disease, however, requires material that represents the diversity within a species, not just one reference strain. For example, the quarantine of US wheat in the late 1990’s was only lifted by having access to diverse strains of the smut fungi, including the species that causes Karnal Bunt.

### **Existing microbial resources**

With extensive holdings of microbial genetic resources, the USDA-ARS has developed a formal policy regarding biological collections.

“The ARS policy is that all scientific objects that make up in part or in whole an ARS scientific collection are publicly funded assets and great care and caution should be taken in regards to their care and preservation” USDA Agricultural Research Service , Scientific Collections Management and Access Policy, September 2014

This policy establishes a sound foundation for management of valuable microbial resources and establishes protocols for evaluation of the status of biological collections. Living collections are a special type of biological collections and include all of the resources in the National Plant Germplasm System (NPGS) and National Animal Germplasm System (NAGP). As such, microbial resources are a subset of all of the USDA’s living collections (Table 1).

Table 1. USDA Public Living Microbe Collections

Title	Acronym	Location	Holdings	Number of strains
ARS Culture Collection	NRRL	Peoria, IL	Bacteria	19,000
ARS Culture Collection	NRRL	Peoria, IL	Fungi	65,000
ARS Collection of Entomopathogenic Fungal Cultures	ARSEF	Ithaca, NY	Fungi	8,000
National Rhizobium Culture Collection		Beltsville, MD	Bacteria	7,000
Antimicrobial Resistance Collection		Athens, GA	Bacteria	93,000

With public holdings of nearly 200,000 unique strains, the USDA is a leading agency supporting access to validated microbial resources. Additionally, the NRRL includes a Patent repository, called an International Depository Authority (IDA), under the Budapest Treaty of 1985 and that has special requirements and obligations. The US has two additional IDAs: the ATCC and the National Center for Marine Algae and Microbiota (NCMA).

While the USDA has several plant virus collections for internal research use, there is no public collection of plant virus genetic resources despite their tremendous impact on global agricultural productivity.

In addition to its public collections, the USDA has diverse holdings of biological research collections in support of their internal research programs. These include living and once living reference material and complement preserved plant and fungal specimen collections used in taxonomy and identification (Supplemental Table A).

As part of the NPGS and NAGP, the USDA maintains a back-up facility at the National Laboratory for Genetic Resources Preservation (NLGRP) in Ft. Collins, CO. Beyond diverse plant and animal germplasm, the NLGRP currently holds duplicate copies of several microbial collections, including the NRRL and the ARSEF, as well as collections once supported by the NSF that are used, at least in part, for agricultural research, such as the Fungal Genetics Stock Center, the Phaff Yeast Culture Collection, and the *E. coli* Stock Center. Many of these are endangered, as the NSF no longer provides long-term support to maintain and curate living collections.

Other living microbe collections in the US support research and development in agriculture and they take several forms (Table2).

Table 2. Agriculturally relevant living microbe collections in the USA

Collection name	Acronym	Number of strains	Location	Website
American Type Culture collection	ATCC	>70,000	Manassas, VA	<a href="http://www.atcc.org">www.atcc.org</a>
Fungal Genetics Stock Center	FGSC	26,000	Manhattan, KS	<a href="http://www.fgsc.net">www.fgsc.net</a>
Phaff Yeast Culture Collection	UCDFST	9,096	UC Davis, CA	<a href="http://phaffcollection.ucdavis.edu">phaffcollection.ucdavis.edu</a>
International VA Mycorrhizal Fungal collection	INVAM	1,112	Morgantown, WV	<a href="http://invam.wvu.edu">invam.wvu.edu</a>
Center for Forest Mycology Research Culture Collection	CFMR	13,241	Madison, WI	<a href="http://www.fpl.fs.fed.us/research/centers/mycology/culture-collection.shtml">www.fpl.fs.fed.us/research/centers/mycology/culture-collection.shtml</a>
World Phytophthora Resource	WPC	8,000	Riverside, CA	<a href="http://phytophthora.ucr.edu/">phytophthora.ucr.edu/</a>

Among these, the ATCC holds type strains (for taxonomic purposes), certified reference materials, and manages human health relevant collections under contract with the National Institutes of Allergy and Infectious Disease. The INVAM, UCDFST, and WPC collections focus on biodiversity of a small group of organisms. The FGSC, like the *E. coli* Stock Center, the Chlamydomonas Center, and the Bacillus Genetic Stock Center, holds isolates with mutations from the classical genetics era as well as modern molecular genetic materials serving a specific research community. These research-resource collections distribute materials broadly. Other entities, such as the California Department of Food and Agriculture, maintain research collections, although they are not available for distribution ([www.cdfa.ca.gov/plant/ppd/ppathcoll.html](http://www.cdfa.ca.gov/plant/ppd/ppathcoll.html)).

While there are diverse resources for working with environmental and plant pathogenic fungi, there is no public collection of plant pathogenic or plant associated bacteria or archaea. Several collections of plant-associated bacteria have either been destroyed or were transferred into the private domain (for profit company) in recent years, including collections at Oregon State University, the University of Nebraska-Lincoln, and the University of Hawaii. Most researchers who require validated isolates of bacteria obtain them from the ATCC, where available, from the German National Collection (DSMZ) in Braunschweig, or from the USDA NRRL collection (although this collection does not have staff to update data on isolates to conform to modern

taxonomy). Similarly, most researchers in fungal biology obtain strains from the taxonomically narrow collection of the FGSC (which currently depends on user fees) or from the Westerdijk collection in the Netherlands (formerly the Centraalbureau voor Schimmelcultures), or from the USDA NRRL collection (which limits the number of strains available annually to any one laboratory/institution).

### Impact

Many of the collections described here have documented impact. For research materials, the number of publications citing a collection is a strong metric. The FGSC has been cited in thousands of publications and the most significant publications have collectively been cited over 100,000 times. Strains from the NRRL collection have been used in over 50,000 publications and patents and these are cited nearly 750,000 times. Because support for microbe collections is distributed among government agencies, not all communities receive equal access to validated microbial resources. When the FGSC grant ended after 60 years of continuous funding, the collection moved to Kansas State University. To cover salary expenses, the collection raised its fees from \$10 per strain to \$50 per strain. The impact of this change was a rapid decrease in the numbers of strains requested. Other collections have had to lay off staff, limit the numbers of strains available to individual researchers, or stop distributing material outside of research collaborations.

The collections described here have been essential for several of the recent key advances in the human condition. The NRRL collection was the source of the first high-penicillin producing strain of the fungus *Penicillium*. The ATCC was the source of the temperature-stable DNA polymerase used in the first demonstration of the Polymerase Chain Reaction. Finally, the *E. coli* Stock Center was the source of strains used in the original demonstration of CRISPR repeats.

Industrial biotechnology, synthetic biology, genome biology, and agriculture all depend on access to high quality, validated microbial resources. By maintaining and sharing research resources that allow the product of one project to be available as the starting point of another, living microbe collections advance research, development, and production at all stages.

Many current USDA ARS projects depend on access to validated microbial germplasm, spanning a range across National Programs (NP): Plant Crop Production (NP301, NP303), Nutrition (NP 107), Food Safety (NP 108), Animal Production (NP 101), and Animal Health (NP 103), Soil and Air (NP 212), Biorefining (NP 213), Grass, Forage, and Rangeland Agroecosystems (NP 215), and Sustainable Agricultural Systems Research (NP 216).

According to the Interagency Working Group on Scientific Collections, “We need to keep items collected in the conduct of research because advances in science depend on a strong and cumulative evidence base.” They further state that “Scientific collections provide an excellent return on the taxpayers’ investments.”

## Current practices

In the absence of formal biological collections, researchers exchange materials on a peer-to-peer basis, or isolate new materials from environmental sources. Both present unique issues. In the former case, while the exchange of living microbial resources among researchers often takes place with best intentions, researchers do not have the expertise or facilities to carry out quality control on materials they share. They may have moved on to new areas of inquiry or may prefer not to make materials available to potential competitors. Moreover, researchers may not be aware of technology transfer obligations or regulations that govern shipping of pathogenic, genetically modified, or other potentially hazardous materials.

Researchers who obtain microbial strains from the environment face issues of reproducibility because microbes in nature are not alike. For example, yeast strains for beer or wine production may not be appropriate for bread production and this is borne out by the availability and exchange of hundreds of yeast strains for home brewing and wine making. Studies have shown that isolates of the same species of bacteria or fungi may differ in genome content by as much as one third due to the presence of dispensable or lineage specific DNA. This sub-species variability makes it imperative to use valid, vouchered, material. Strain similarity can be validated by whole genome sequence analysis and this is an important new horizon for insuring research repeatability.

An additional challenge to using microbes from *ad hoc* sources is the diversity of national legislation being enacted around the world in response to mandates of the Nagoya Protocol on Access and Benefit Sharing. While not every country has done so, many have. This means that the age-old practice of using isolates from imported produce may violate legislation in the country of origin.

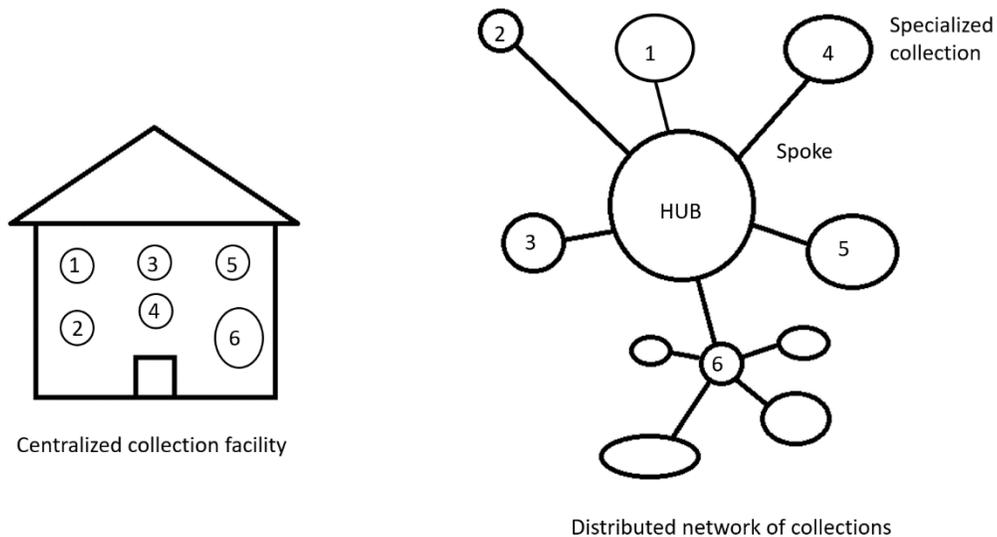
## Specific Recommendations

- Public USDA ARS living collections should be supported at a level that allows them to “effectively and efficiently manage microbial genetic resources”.
  - Curators should enhance the collection by updating taxonomy and developing genome resources
  - Curators should conduct research on preservation and storage technology
  - Curators should have access to staff support in information technology, shared quality control, and regulatory affairs
- Resources for bacterial and archaeal research and taxonomy should be expanded
- Collections should develop and maintain high-quality, easily-accessible databases

- ARS scientists who maintain and curate microbial collections should include a transition plan in their project plan for the maintenance of their collections after retirement or separation from the agency.
- Diverse collections, including university based collections, should be incorporated into a network or center.
  - This effort should include cross-agency cooperation
  - These collections could be in a central facility or distributed to capitalize on research expertise at universities or research facilities
  - Alternative structures are shown in Supplemental Figure 1 and the USDA should evaluate
- The USDA National Laboratory for Genetic Resources Preservation in Ft. Collins, Colorado should have an explicit mandate and appropriate funding for a curator and support staff to conduct research and service activities for microbial resource preservation and distribution
- Microbiome resources for crop plant and animal production should be cataloged and made available through formal microbial repositories
- A specific mandate for inclusion of biocontrol organisms in the NRRL collection should be established
- A public repository of plant virus resources including viruses and infectious clones should be established at the USDA ARS NRRL collection.

Supplemental Figure 1. Alternate structures for collection support. A centralized collection has multiple resource types in one facility while a Spoke-and-hub relies on a central hub for information technology, quality control, and regulatory affairs. The different specialized collections can be focused on one group of organisms (1 – 5) or, like the USDA ARS NRRL, include taxonomically diverse holdings and a patent collection (IDA).

Currently, the USDA National Plant Germplasm (<https://www.ars-grin.gov/npgs/>) system employs a distributed network system while human health (<https://www.beiresources.org/>) or invasive pest resources (eg, <https://www.ars.usda.gov/northeast-area/frederick-md/foreign-disease-weed-science-research/>) are maintained in centralized facilities.



Supplemental Table A. Other USDA Living Pest Collections (compiled from USDA documents and web resources)

Title	Location	Holdings	Number of strains
BARC Living Fungal Collection	Beltsville, MD	Fungi	11,000
Entomopathogenic Nematode Collection	Byron, GA	Nematodes	50
Florida Meloidogyne Collection	Ft. Pierce, FL	Nematodes	?
Orthopteran Microsporidia Collection	Sidney, MT		350
Microsporidia in Mosquitoes and Fire Ants Collection	Gainesville, FL		5
Microsporidia and Gregarines in Stored Products Pests Collection	Manhattan, KS		6
BARC Collection of Insect-Pathogenic Viruses	Beltsville, MD	Virus	2,600
Biological Control of Insects Unit Virus Collection	Columbia, MO	Virus	20
Pest Management Unit Grasshopper Virus Collection	Sidney, MT	Virus	?
Quarantine Virus/Viroid Collection	Beltsville, MD	Virus	60
Potato Virus Y Isolates Collection	Ithaca, NY and Aberdeen, ID	Virus	3,000
Exotic Pathogens of Citrus	Beltsville, MD	Virus	90
Ornamentals-associated Plant Virus Collection	Beltsville, MD	Virus	60
Vegetable and Sugarbeet Virus Collection	Salinas, CA	Virus	?
Citrus Tristeza Virus Collection	Parlier, CA	Virus	125
Cereal Virus	Fargo, ND	Virus	?
Small Fruit Crops-Plant Virus Collection	Corvallis, OR	Virus	250
Specialty Crop-Associated Plant Pathogen Collections	Corvallis, OR	Virus	97
Arthropod-borne Viruses	Laramie, WY	Virus	?
Avian Viruses	Athens, GA and East Lansing, MI	Virus	2,000
NACD Virus Collection	Ames, IA	Virus	1,100

**For more information:**

Biological Collections: Ensuring Critical Research and Education for the 21st Century.

US National Academies of Science, Board on Life Sciences, 2020

Press release: <https://www.nationalacademies.org/news/2020/09/critical-to-scientific-discovery-and-innovation-biological-collections-need-strategy-action-center-and-increased-investment>

Full Report: <https://www.nationalacademies.org/our-work/biological-collections-their-past-present-and-future-contributions-and-options-for-sustaining-them>

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Preserving US microbe collections sparks future discoveries. 2020. Journal of applied microbiology 129 (2), 162-174.

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Public Microbial Resource Centers: Key Hubs for Findable, Accessible, Interoperable, and Reusable (FAIR) Microorganisms and Genetic Materials. 2019. Appl Environ Microbiol 85:e01444-19. <https://doi.org/10.1128/AEM.01444-19>.

The U.S. Culture Collection Network Lays the Foundation for Progress in Preservation of Valuable Microbial Resources. 2016. Phytopathology 106:532-540.

<https://apsjournals.apsnet.org/doi/10.1094/PHYTO-02-16-0074-RVW>

The US Culture Collection Network (an NSF sponsored Research Coordination Network):

<http://www.usccn.org/Pages/default.aspx>