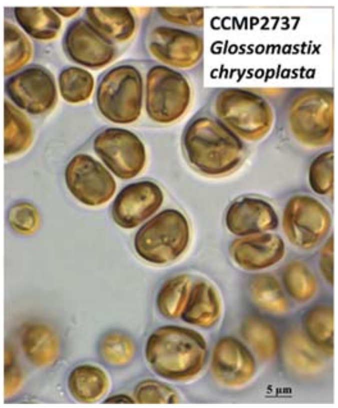
Algal Genetic Resources: Agricultural Crops and Beyond

June, 2022

USDA National Genetic Resources Advisory Council




This report was drafted by the team below with input and final approval by the NGRAC

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Executive Summary

Natural stocks of algae have been a source of food, feeds and fertilizers for coastal communities for millennia. Modern biotechnological use of algae in human systems has been rapidly expanding in scope and diversifying in application over the past few decades, in no small part through the maintenance and use of algae as a genetic resource. This importance of algae is underscored in the 2018 U.S. Farm Bill that, for the first time, recognized the need for and support of algal crop production systems. As with agricultural, livestock, and bio-industrial processes, ready access to well-characterized and evaluated (i.e., phenotyped and genotyped) strains is an essential foundational infrastructure resource. Algae culture collections are presently the primary U.S. infrastructure that maintains and distributes this authentic, well-characterized and validated biological material. The U.S. currently has five publicly assessible algae culture collections, which have developed independently over decades, primarily to support academic research in taxonomy, biodiversity, and ecology. The rapid diversification and expansion of both basic and applied algae research, particularly in this era of synthetic biology and genetic engineering advancements, highlights the need to support strategic investment in expansion, stabilization and integration of these existing algae culture collections and related algae knowledge centers into a formal Algal Library and Genetic Information Network (ALGINet). The lead author of this report to the USDA was also the lead Principal Investigator on a National Science Foundation Mid-Scale 2 Research Infrastructure proposal focused on developing a national algal germplasm resource. Recommendations that emphasize the opportunity to build upon existing resources to meet future needs are summarized as:

1. *Evaluate the costs and benefits of a distributed (spoke-and-hub) versus centralized U.S. national algal genetic resource system and its interactions with and connections to the existing USDA-ARS.* This should include assessments of how current algal collections align with current and future USDA priorities, leveraging knowledge from extant systems such as NPGS, and develop guidelines for strategic growth.

2. *Expand support for algal culture collections to a level that ensures their current stability and responsiveness to future developments.* This should include expansion in physical infrastructure, methodologies and management systems (e.g., expanded cryopreservation), and operational practices (e.g., personnel training, information technology and regulatory guidance).

3. *Develop and/or bolster targeted collaborations between U.S. federal agencies (e.g., USDA, NSF, DOE), land and sea grant universities, tribes and NGOs engaged in algal research utilization to effectively and efficiently manage future funding for, demands on and opportunities for algal collections.* This should include a strong focus on standardized collection facilities, methods, management practices and personnel training.

4. *Support development and compilation of uniform information systems across existing algae collections in support of conservation, research and commercialization efforts.* This should specifically focus on improving access to distributed phenotype and genotype data sources.

Societal Impacts and Valuation of Algae

Algae are non-flowering aquatic 'plants' that can be generally categorized into microalgae, microscopic single-celled plants, and macroalgae, macroscopic multi-cellular plants often referred to as seaweeds. While both algae and terrestrial plants carry out photosynthesis, algae lack true stems, roots, leaves, and vascular tissue found in terrestrial plants. Algal taxonomy and ecology have been studied for over 250 years leading to important scientific discoveries from their own evolutionary relationships to the rise of multi-cellular terrestrial plants from green algae lineages to genomic and phenotypic adaption to environmental extremes. However, algae have been part of human and animal nutrition for millennia, with the use of algae in diverse modern agricultural and biotechnology applications being comparatively recent. Algae directly serve as a source of food and nutrition (e.g., essential fatty acids) for humans and animals, which has led to their inclusion in the U.S. Farm Bill, but their uses and societal relevance go far beyond agricultural crops (**Figure 1**).

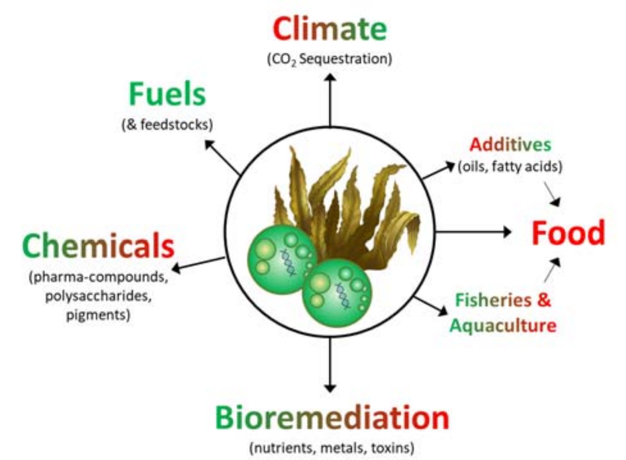

Figure 1. Applications and societally relevant activities to which algae have made contributions. Red: macroalgae and Green: microalgae as a feedstock source in the specific area.

As aquatic 'plants', algae support natural fisheries and aquaculture worth many billions of dollars worldwide. Aquaculture of mollusks (oysters, mussels, clams, abalone and others) and fishes represents a quarter of global seafood production, and algae are increasingly used to improve sustainability of these specific aquaculture practices through co-culture. On its own, wild harvest and aquaculture of macroalgae, of which aquaculture represents more than 90%, supports more than \$13 B in global business annually primarily as a food staple (**Figure 2**). Macroalgae aquaculture has grown exponentially in the U.S. over the last decade amounting to more than \$0.2 B annually. While U.S. macroalgae aquaculture production lags other countries in Asia and Oceania where macroalgae has been a more significant component of their diet for a longer period of time, there is significant potential for growth with some economic analyses suggesting continuation of 8% year-over-year growth in this sector.

Commercial microalgae biomass production for food systems, albeit smaller in magnitude than macroalgae and dominated by production of *Spirulina*, still supports \$0.5 B in global direct-use business due to the greater value per unit biomass. This sector is however, growing rapidly as it interacts with agri-business sectors such as livestock and crops. Microalgae in particular are used as additives in livestock feed formulations (e.g., ruminants, swine, poultry and pets) as a sustainable, and non-competing, source of proteins, essential fatty acids and oils, and natural colorants. Natural algal extracts, both micro- and macroalgae, are increasingly being used as biostimulants for terrestrial agricultural crops, and to reduce enteric methane emissions in ruminant livestock, leading to improved sustainability in both production systems. The integration of algae adds substantial value to these trillion-dollar industries by improving productivity and reducing losses, thus leading to more sustainable industries.

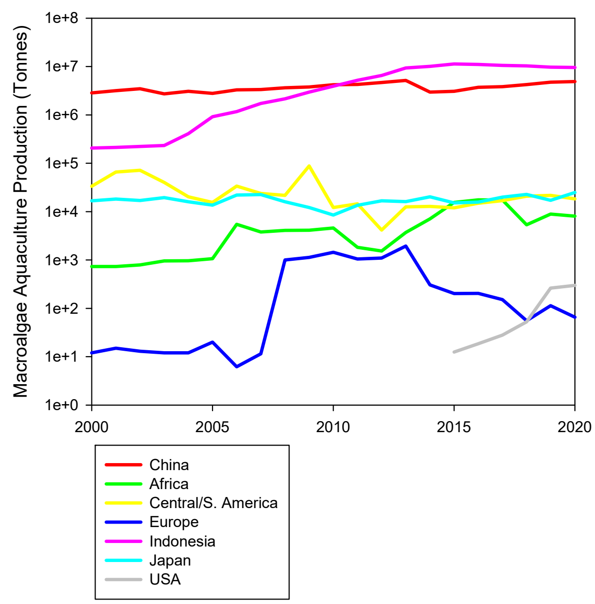

Figure 2. Macroalgae biomass production over time by country/region. Data from FAO 2020.

In addition to direct and indirect food resources, algae have received significant attention in the past two decades as biofuel feedstocks, and as sources of diverse high value, sustainably produced molecules and chemicals (e.g., agar, carrageenan, alginates, fucoidans, omega-3 fatty acids, and phycobiliproteins and other pigments) that can replace synthetic versions in food production systems. It is in these sectors where microalgae are more diversified than macroalgae (**Figure 3**). For example, the global omega-3 fatty acid market is roughly \$35 B, with omega-3 fatty acids derived from cultured microalgae representing 10% of this market. Stimulated by advances in synthetic biology and genetic engineering, algae are also being used in other creative ways, such as cell factories for the sustainable production of plastic products such as sandals,

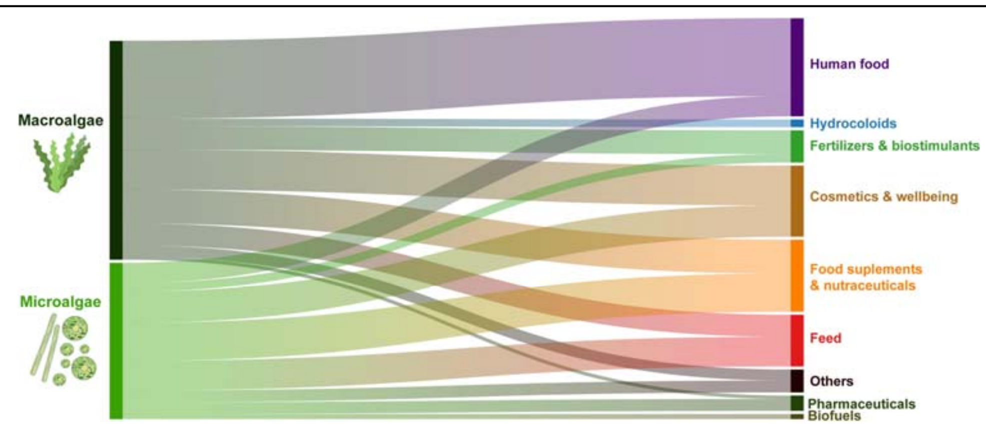

Figure 3. Distribution of commercial biomass applications for macroalgae and microalgae companies in Europe by major use sector (modified from Araujo et al. 2021).

surfboards and straws. Algae also play an important role as integrated biorefineries in biotechnology and the pharmaceutical industry as they are increasingly recognized as a source of novel antioxidant, antibiotic, antifungal, anticancer and antiviral compounds.

In addition to the myriad ways in which algae contribute to human food systems and serve as raw materials for industrial processes, algae can also be directly integrated in solutions to provide essential, societally relevant ecosystem services such as carbon dioxide removal (CDR) thus mitigating some of the harmful effects of climate change. For example, microalgae are currently used to 'scrub' carbon dioxide from flue gas streams at a range of scales, and macroalgae are increasingly grown and 'sunk' to the deep ocean removing carbon dioxide from natural aquatic systems. Algae are also used in the transformation of toxins, chemicals and heavy metals (bioremediation) from industrial processes. Similarly, algae are cultured to treat wastewater, removing excessive nitrogen and phosphorus (nutrient bioextraction) that have been implicated in the expanded prevalence of harmful algal blooms. While less than one percent of known microalgae species produce harmful toxins, they can result in disproportionately large ecosystem and economic impacts, and for this reason take on increased scientific value. These ecosystem services provided by algae are difficult to value directly, but for an approximation of scale, the U.S. spends hundreds of billions of dollars each year on non-algae-related carbon capture and wastewater treatment operations and infrastructure.

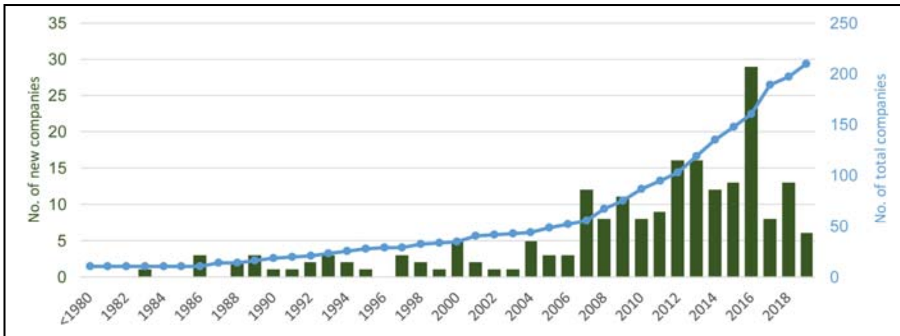

Figure 4. Growth of algae producing companies in Europe (modified from Araujo et al. 2021).

Algae affect the daily lives of most Americans, often without our knowledge. Into the future, this diverse array of algae-based science and application research will likely continue to expand in both production volume and economic value through creation of new companies as has been seen in Europe (**Figure 4**). The continued development of genetic tools to extract value from algal genetic resources is likely to amplify this trend. Realization of this growth potential and the use of algae in agriculture, aquaculture, and industry requires sustained infrastructure resources, specifically collections of diverse, well-characterized, high-quality and readily available algal cultures.

Algae in the U.S. Farm Bill

It is only recently that large scale macroalgae aquaculture has been developed for food in the U.S., thus increasing the focus on algae as a 'crop'. The passage of the U.S. Farm Bill in 2018 (Public Law No. 115-334) was the first time that algae (either microalgae or macroalgae) were explicitly included in agricultural legislation. The Algae Agriculture Research Program (P.L.115-334 §

7029.17) supports the development and testing of algae production systems to produce algae as a 'crop', although precise funding mechanisms (e.g., NIFA, AFRI, special projects) remain to be specified. Consistent with this inclusion, algae are also included under the Biomass Crop Assistance Program (P.L. 115-334 § 9010), which provides further financial assistance to establish, produce and deliver biomass feedstocks. This designation of algae as a crop aligns with its high societal relevance, and it will likely further stimulate rapid expansion of scientific and applied research and development of commercial products involving algae in human food systems, algae-based livestock feed, feedstocks for biofuels, and other domestic and global industrial sectors. With the rapid development of synthetic biology tools specific to algae, researchers and stakeholders can tune the characteristics of specific algal strains to drive innovation in agriculture and aquaculture sectors, but these advances are wholly dependent upon having ready access to validated wild type and modified strains of algae with known and well characterized genotype and phenotype characteristics. Algae collections hold these wild type and modified strains, and support of a national algae germplasm system would enable diverse researchers to engage further down the path from application to commercialization. ***The imperative for an organized and responsive national algae collection and genetic resource strategy cannot be overstated, so as not to miss important and potentially game-changing opportunities for the U.S.*** Indeed, just as the formation of the National Plant Germplasm System in the 1970's stimulated development of a sustainable agriculture system and the 'green revolution', formation of a national germplasm system for algae will enable this next generation of the green revolution.

Current Status of U.S.-based Living Algae Resources

In the U.S., there are several publicly accessible living algae germplasm collections that curate, maintain, and distribute a wide diversity of marine and freshwater algae strains (**Table 1**). These algae collections are distinct from the more numerous herbaria and other non-living plant collections. While there is some overlap in the holdings between these algal collections, each collection has a unique and intentionally narrow focus that collectively allows general coverage of the broad diversity of algae. An outcome of this narrow focus is that these publicly accessible collections are heavily skewed to microalgae holdings. Although UTEX and NCMA do hold some macroalgae strains, strategic expansion to include macroalgae is currently resource limited (i.e., financial and infrastructure). In contrast to these public access, microalgae collections, macroalgae germplasm collections are generally found associated with university research programs and are made 'publicly available' only through collaborative research relationships. These macroalgae research collections are currently experiencing a heightened level of risk and are prone to loss due to a lack of sustainable grant-based funding models, and thus represent a potentially significant loss of prior scientific investment. The magnitude of this potential loss is difficult to evaluate as there is no central resource listing these university research collections and their holdings. Indeed, it was this exact problem that led to the consolidation of U.S. marine microalgae research collections in the early 1980's and the creation of the NCMA (at the time called the Culture Collection for Marine Phytoplankton, CCMP). The ability of extant algae collections to absorb potentially orphaned macroalgae collections is quite limited as they themselves still struggle to achieve sustainable financial models. This is particularly acute as the financial models for the UTEX, NCMA, and ARC collections are a balancing act of competitive grant-based funding, sales of algae and algae products, and ancillary funded projects to close their annual budgets all of which are much less than 1 million dollars per year.

Table 1. U.S.-based public algae collections that support basic and applications-based research.

Collection (year started)	Collection Name	Description of holdings	URL
ATCC (1925)	American Type Culture Collection	Algae collection (63 strains of primarily green algae)	https://www.atcc.org/
UTEX (1953)	University of Texas Culture Collection of Algae	More than 3,000 unique strains of primarily freshwater microalgae and 174 macroalgae strains comprising 6 kingdoms, with 1,469 named species spanning 565 genera	https://utex.org/pages/discover-algae/
CRC[§] (1979)	*Chlamydomonas* Resource Center	More than 3,000 physiologically unique strains of *Chlamydomonas reinhardtii*	https://www.chlamycollection.org/
NCMA (1981)	National Center for Marine Algae and Microbiota at Bigelow Laboratory for Ocean Sciences	Roughly 3,000 unique isolates of primarily marine microalgae and 1,488 macroalgae strains comprising 52 families with > 500 genera and >725 named species.	https://ncma.bigelow.org/
ARC (2016)	Algal Resources Collection	353 strains of algae from 6 groups, with a strong focus on algae that can produce harmful compounds	http://www.algalresourcescollection.com/

[§] *Chlamydomonas* is a model microalgal system. This collection holds the 'genetic resource' for *Chlamydomonas*, including all the various strains with different physiological traits.

Diversification: Estimates of algae diversity, both micro- and macroalgae and freshwater and marine, range from 30,000 to > 1 million, although most scientific evidence suggests this number is ca. 70,000. Given the comparatively new focus on manipulation of algal genetics, U.S.-based algal germplasm collections hold primarily wild-type algae strains thus reflecting a portion of the naturally occurring algal biodiversity. Collectively the U.S. algal collections hold ca. 6,000 microalgae strains and ca. 2,000 macroalgae strains, representing roughly 10% of the microalgae and macroalgae that have been scientifically described. In addition to U.S. algae collections, there are ca. two dozen algae collections globally, determined from the World Federation of Culture Collections database, each with varying degrees of public access. Significant access to the holdings of these collections can be challenging due to the constraints of the Nagoya Protocol, part of the Convention on Biological Diversity, the existence of only informal relationships between global algal collections, and more fundamental challenges with the global shipping of live algae. Development of a U.S. algae germplasm system should focus on existing U.S. resources rather than be dependent upon other international algae culture collections. This will require establishing decision trees for 'intelligent' and strategic diversification of collections to make them more representative of present and future needs.

Capacity: While existing U.S. algal germplasm collection infrastructure, resources, and workforce have slowly grown over time, their capacity has been outpaced by relevant scientific advances and initiatives. As a matter of scale, individual researchers can make more new "strains" from a well characterized wild type algae with single gene edits (using CRISPR/CAS9 technology) *in just a few months* than the accumulated holdings of U.S. algae collections over the past 50 years; clearly not all genetically engineered strains can or should be preserved in germplasm collections. Unambiguous and strategic regulations and/or guidelines to moderate a decision-making process for accessioning strains into, or removing them from, germplasm collections remain to be written. In addition, focused federal programs, e.g., ARPA-E Mariner Program, have led to the collection of thousands of new macroalgae isolates that currently have no established and sustainable support infrastructure to ensure their security beyond the end of the program. Existing algal collections operationally do not have the capacity to sustainably accommodate any large-scale expansion in demand for providing and storing algal strains associated with academic, commercial and agricultural industries that may be stimulated by the inclusion of algae in the U.S. Farm Bill.

Storage: While expanding capacity poses a challenge in terms of availability of skilled personnel and infrastructure capacity to maintain these germplasm collections, it presents a great opportunity to enable scientific advances and development of more appropriate methods for algal germplasm collection management. While there are other methods for long-term storage of microbes (e.g., lyophilization), cryogenic preservation is the preferred method for algae. Cryopreservation of algal strains limits the possibility of genetic drift, and potential loss of desirable traits and economic value, that might otherwise be observed in perpetual cultures. However, successful cryopreservation remains largely empirical, requiring the testing of multiple cryoprotective agents and ranges of concentrations. As part of any capacity expansion plan, algal collections need to be supported in their efforts to increase cryopreservation infrastructure as a key collection management tool. Translation of existing high-throughput cryopreservation knowledge and infrastructure developed in other genetic resource sectors to algal collections will accelerate the response to this need.

Information Infrastructure: Investment in enhancing and expanding algal germplasm collection capacity, management tools and infrastructure is only part of the challenge; U.S. scientific and commercial researchers want and have come to expect easier access to uniform information on algal phenotype traits and genetic information, genome-to-phenome, to maintain the rapid pace of algal innovation. One need only look to the *Chlamydomonas* Resource Center to see the value of having a catalog that not only defines algae by physiological traits, but also holds many physiological variants from the wild type strain. Currently each U.S. algae collection uses a different platform for compiling its catalog of strains, which makes cross platform searches challenging. Further, there is no uniform database format, with some collections cataloging strains by their physiological traits and others by their ecological characteristics. As with most biological entities, there are a number of unique characteristics that should be included in databases such as strain type, optimum culture media formulation, cell size and biovolume information, cell and biomass yields under optimum growth conditions, toxicity and agronomic characteristics to leverage the most value for agricultural and commercial applications. There are several national and international activities (e.g., U.S. Culture Collection Network, Scientific Collections International, Biodiversity Collections Network and Global Biodiversity Information Facility) that function as clearinghouses for diverse aggregations of data that can serve as a starting point for the development of a consistent and informative platform specific to algae germplasm collections. There is also the open-source Germplasm Resource Information Network (GRIN-Global) system that has been designed specifically for integrating a wide array of genetic resource information into a single user-friendly interface. The range of existing platforms provides a solid foundation for evaluation and then building an appropriate algal genetic resources database system.

A Look to the Future

In the future, algal collections face many questions, but perhaps the most important is will they be proactive or reactive in their decisions as holders of algal genetic diversity and resources? In the past and present, algal collections, for the most part, have accessioned strains that represent algal biodiversity that foundational science has found appropriate for curation. With aquatic systems changing rapidly, what is the role of algal collections and an algal genetic resource system in looking forward and proactively participating in the conservation of vulnerable species, or species with unique traits? As a specific example, over the last five years California Bull Kelp populations have been reduced by 90% associated with anomalously warm oceanographic conditions. With the loss of these populations comes the loss of a range of valuable ecosystem services (e.g., nursery habitats for other aquatic organisms, mitigation of coastal erosion). While germplasms cannot mitigate loss of genetic diversity for aquatic ecosystems, how do these indirect valuations factor into a balanced decision-making process regarding development of an algal genetic resource system that can be responsive to future scenarios and priorities?

Even as we are seeing the loss of natural algal genetic resources from aquatic systems, algae are also increasingly viewed as a promising pathway to address many of society's greatest challenges from the sustainable production of existing and new food systems to mitigating the worst impacts of climate change. References to the use of algae in human food systems are increasing in frequency, as are predictions of its future value. In November 2020 a new report suggested that algae-based protein alone will grow to a market value of $1.2B by the year 2027, and wide-ranging and creative commercialization efforts will only positively affect the value of algal products. This growth of algal products and applications is a global phenomenon. As the use of sustainably sourced biological resources rises to meet global challenges (e.g., protein sourcing, carbon sequestration, water treatment), this globalization requires continued diligence to international treaties such as the Nagoya Protocol that governs access to and benefit-sharing from genetic resources. As with any commercial activity, realization of revenue requires investment. The foundation represented by existing algae collections will accelerate the return on investment.

Through engagement with diverse collections such as the NSF-supported bacterial and fungal Genetic Stock Centers, the U.S. Culture Collection Network, the World Federation of Culture Collections and the NPGS, algal collections have a good blueprint to improve management, curation, quality control, and support expansion of diverse applications of algae in industry, aquaculture and agriculture into the future.

Safeguarding the known and untapped genetic resources of algae presently in U.S. public algal collections, and building capacity for their strategic expansion in the future, is of utmost importance for the U.S. to continue gaining scientific and financial benefit from algae in the rapidly expanding global 'blue economy' and a changing world.

Specific Recommendations

Outlined below are recommendations necessary to comprehensively maintain, strategically expand and connect U.S. algal germplasm collections and associated algal knowledge centers as the foundation of an algal genetic resource; the Algal Library and Genetic Information Network (ALGINet). These recommendations will also ensure the future stability and viability of such enterprises in conservation of algal diversity and to support the continued growth of algae into diverse economic sectors, including food and feed crops. We note that some of these recommendations will be sequential, while others may be concurrent. If it is decided to act on these recommendations, a more detailed implementation plan, specifically including a phase-gate process, would need to be created.

1. ***Evaluate the costs and benefits of a distributed (spoke-and-hub) versus centralized U.S. national algal genetic resource system and its interactions with and connections to the existing USDA-ARS. USDA should stand up a committee of experts, including scientists and stakeholders (e.g., similar to the standing Crop Germplasm Committees that are part of the NPGS) to:***
   a. Provide a detailed evaluation of how algal collections currently support agriculture, and how future efforts align with USDA priorities. This should also include advice on future collection priorities.
   b. Evaluate the benefits and costs of aligning the algal genetic resource system to the broader National Plant Germplasm System.
   c. Establish transparent guidelines and decision trees for inclusion of wild-type species and strains for conservation, basic research, and commercial application.
   d. Establish transparent guidelines on the responsible handling of genotypes arising from genetic engineering and/or gene editing efforts. This should include, but not be limited to, guidelines/strategies on which genotypes to accession and which should be available for distribution.

2. ***Expand support for algal culture collections to a level that ensures their current stability and responsiveness to future developments. USDA should:***
    a. Develop mechanisms for funding operational support of algal collections to appropriately maintain, curate and distribute the diversity of algal genetic resources currently held and those accumulated in the future.
    b. Provide support for the expansion of storage infrastructure at algae collections to accommodate newly discovered and genetically modified algae strains, specifically those associated with agriculture and aquaculture innovations.
    c. Provide support for the implementation of high-throughput cryopreservation techniques at algal collections as part of developed collection management plans.
    d. Establish a process to regularly identify, evaluate and implement investments in personnel (e.g., curators with appropriate knowledge and training) and operational support (e.g., Information Technology, regulatory guidance) priorities to ensure operational efficiency.

3. ***Develop and/or bolster targeted collaborations between U.S. federal agencies (e.g., USDA, NSF, DOE), Land and Sea Grant universities, tribes and NGOs engaged in algal research utilization to effectively and efficiently manage future funding for demands on and opportunities for algal collections. USDA should support:***
    a. Development of standardized collection facilities and methodologies within algae collections to reduce operational costs, enable future science advances, and improve responsiveness to national level priorities.
    b. Develop on-line training content specific to algal germplasm bank management and strain curation. The online course "Plant Genetic Resources – Genomes and Genebanks" (https://www.online.colostate.edu/courses/AGLL/AGLL3501.dot) may serve as a model.
    c. Continued Development of formal training courses and workforce development opportunities offered through algae collections to prepare for present and future workforce needs. For example, NCMA currently offers an in-person training short course on basic algal isolation, curation and maintenance techniques.

4. ***Support development and compilation of uniform information systems across existing algae collections in support of conservation, research and commercialization efforts. USDA should support:***
    a. An evaluation of the benefits and limitations of existing genetic resource information systems (e.g., GRIN-Global), and develop and implement a one-stop algae genetic resource information system linked to existing algae collection holdings to reduce duplication of effort.
    b. Development of a pipeline to integrate knowledge of algal physiological and ecological traits (for example production, yield and organoleptic flavors, key uses, growth behavior, ease of use) into the new algal genetic resource database.

Suggested Additional Reading:

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