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Microbiological Collections in Brazil: Current Status and Perspectives

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Abstract: As part of a Ministry of Science, Technology, and Innovation initiative, the Brazilian Societies of Botany, Microbiology, Virology, and Zoology conducted a comprehensive evaluation of biological collections in Brazil. This assessment aimed to gather insights into the current state of these collections, with the goal of providing support for future public policies, including financial subsidies and prioritization policies. In this context, we present the findings related to microbiological collections, essential to ex situ biodiversity conservation and crucial in supporting research, development, and innovation. A survey was distributed to public and private institutions across Brazil, yielding responses from 168 microbiological collections representing 79 different entities. Notably, 73 of these collections are affiliated with public research institutions and universities, underscoring the State's pivotal role in preserving and safeguarding Brazilian microbial diversity. The primary taxonomic groups encompass bacteria (found in 70.24% of collections) and fungi (comprising 52.98% of collections), sourced from diverse Brazilian ecosystems and biomes, including those that contain several type strains. Furthermore, the collections preserve microorganisms harboring biotechnological potential applicable to environmental protection, public health, industry, and agribusiness. Despite these promising economic and biotechnological prospects, our meticulous data analysis has revealed significant limitations and vulnerabilities, especially regarding physical infrastructure and human resources, emphasizing the urgent need for interventions to guarantee their sustainability.

Keywords: culture collections; biological collections; biodiversity; conservation

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1. Introduction

According to the Convention on Biological Diversity (CBD), Brazil is the most biodiverse country in the world [1], and as Brazilian researchers describe new species and report discoveries about species distribution in Brazilian biomes daily, a large proportion of microbial species in the country remain undescribed as of yet.

Despite the fact that a large portion of its economy is derived directly from biological resources, Brazil has invested very little in promoting studies in biological diversity. In recent years, we have witnessed the effects of climate change, fires, and environmental disasters in Brazilian biomes as key drivers behind the loss of biodiversity, which has also been attributed to changes in the intensity of land use and overexploitation by agriculture [2]. This progressive loss of natural habitats places new demands and challenges on sustainability in agriculture and soil biodiversity conservation. All related studies and

applications rely on biodiversity conservation strategies, which must be carried out in situ and ex situ.

In fact, culture collections play a pivotal role as indispensable biorepositories, contributing significantly to biodiversity preservation and serving as crucial assets for various sectors such as health, environmental, agribusiness, and industry. The Convention on Biological Diversity (CBD) has played a major role in driving a paradigm shift on the global stage by emphasizing the importance of biological collections. Consequently, these collections have become integral components of the research infrastructure of all nations. In the context of Brazil, the recognition of this importance has been enforced in the current legislation through Law 13,123/2015, which addresses access to genetic resources, the associated traditional knowledge, and the equitable sharing of benefits. As a result, strategic investments in microbial collections emerge as substantial directives for the sustainable utilization of biodiversity and the safeguarding of national sovereignty. Given the limited availability of financial resources, it is imperative for the Brazilian government to strategically allocate these funds, prioritizing needs sensibly. However, achieving such a rational and sustainable utilization of financial resources depends on a comprehensive understanding of the reality, challenges, and requirements of each Brazilian collection. Currently, we find ourselves with more inquiries than solutions. For instance, questions such as the number and location of Brazilian microbial collections, the types of institutions housing them, and whether all biomes and taxonomic groups are adequately represented remain unanswered. Additionally, details such as which collections house deposits of type strains and the preservation methods they employ are unknown. An in-depth exploration of the strengths, weaknesses, and needs of these collections is essential to ensure the secure preservation of biodiversity.

To address these inquiries and gain a comprehensive understanding of the overall status of biological collections in Brazil, the Ministry of Science, Technology, and Innovation has promoted a collaborative effort involving the Brazilian Societies of Botany, Microbiology, Virology, and Zoology in a campaign to conduct an extensive survey on Brazilian biological collections. The survey encompassed three major phases: (1) the development and validation of a comprehensive questionnaire covering various aspects of the organization and management of biological collections; (2) the prospection of curators and professionals potentially maintaining collections in public and private institutions, soliciting their input through the prepared questionnaire; (3) the compilation and analysis of the collected data. In the present study, we focus on the results related to microbiological collections, offering an overview of their current status. Additionally, we discus essential strategies aimed at the preservation of Brazilian microbial biodiversity.

2. Materials and Methods

2.1. Survey Structure

A comprehensive survey was built to understand the status and organization of the Brazilian microbiological collections properly. The survey comprised 92 different questions organized into nine sections about different aspects related to the organization and management of collections: (1) identification, (2) characterization, (3) infrastructure, (4) personnel, (5) access, (6) digitalization, (7) quality management, (8) collection management, and (9) priorities.

For all nine sections, most of the questions provided previously defined closed alternatives for the answers, with a few exceptions allowing for open answers. For questions related to ordinal variables, a unipolar scale (from “minimum” to “maximum” and from “worst” to “best”) with an odd number of alternatives was employed.

The survey was initially reviewed and validated by 10 curators from representative microbiological collections from different kinds of institutions and Brazilian regions. These curators evaluated the feasibility and completeness of the survey, suggesting corrections and changes to be made to the final version. The applied survey containing the questions and options provided can be found in Supplementary Materials.

2.2. Assessing the Microbiological Collections

A preliminary list with already known and established microbiological collections was built based on data collected from the main Brazilian public databases, such as the Brazilian node of the Global Biodiversity Information Facility, SiBBr (Sistema de Informação sobre a Biodiversidade Brasileira, https://collectory.sibbr.gov.br/collectory/, accessed on 28 March 2022), and SpeciesLink (https://specieslink.net/search/, accessed on 28 March 2022). Additional collections were also contacted based on data provided by the Brazilian Society of Microbiology and Brazilian Society of Virology, as well as lists compiled by national, regional, and international federations and networks such as the World Federation for Culture Collections (WFCC), *Federación Latinoamercina de Colecciones de Cultivos Microbianos* (FELACC), and *Napi Taxonline—Rede Paranaense de Coleções Biológicas*. Moreover, to reach potential unknown collections that were still absent from official databases and lists, the survey link was also sent to researchers from Brazilian public and private universities and research institutes and advertised on social media platforms such as Facebook, Twitter, and Instagram.

2.3. Data Analysis

Answers received between April and June 2022 were manually processed and reviewed before further analyses. All answers for all 92 questions were individually assessed, searching for potential mistakes and inconsistencies. After revisions and corrections, the results were further analyzed and processed in Microsoft Excel, and final plots were produced in R [3] using the tidyverse package.

3. Results

3.1. Public Institutions in Brazil Are Responsible for the Vast Majority of Microbial Collections

The survey was sent to public and private institutions in Brazil and was answered by 168 microbiological collections from 79 different institutions. Among these institutions, 73 (92.4%) comprise public research institutions and universities (Figure 1).

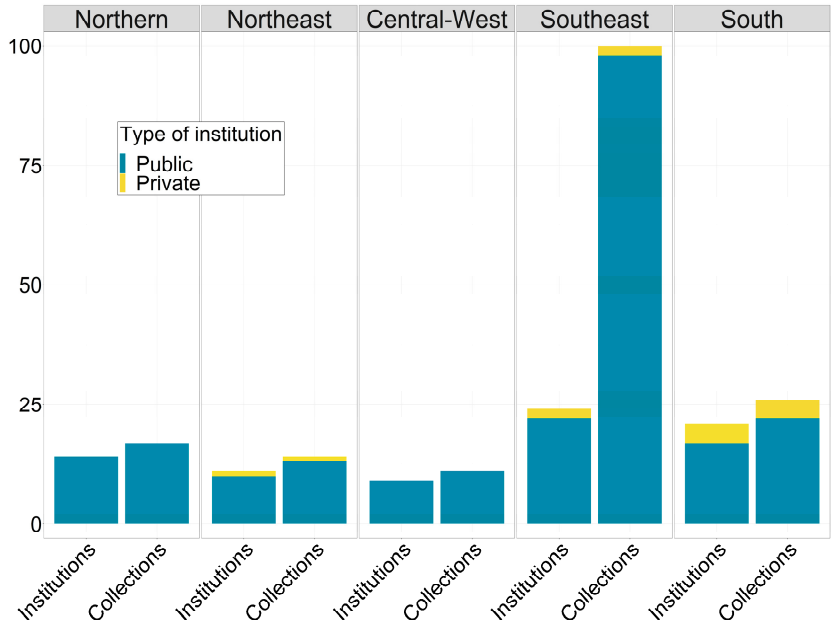

Figure 1. Brazilian microbiological collections are mainly located in public institutions. Stacked bar plots showcasing the distribution of public (blue) and private (yellow) institutions along with microbial locations and their regional distribution.

Brazil is divided into five regions: North, Northeast, Central-West, Southeast, and South. The 27 states of the federation are distributed in these five regions. There is apparent

heterogeneity between these Brazilian regions, with the Southeast region alone accounting for 59.5% of the Brazilian microbial collections (Figure 1). On the one hand, it is the most populous region of Brazil (it concentrates 42.06% of the Brazilian population); on the other hand, it is home to only part of one of the six Brazilian biomes (Atlantic Forest).

3.2. Most Collections in Brazil Have Bacteria and Fungi

The main taxonomic groups preserved in Brazilian culture collections are bacteria (present in 70.24% of the collections) and fungi (52.98%) from different Brazilian ecosystems and biomes, including ex situ preservation of several type strains (Figure 2). Bacteria and fungi are usually the most abundant taxa in microbial culture collections worldwide [4,5]. As expected, most Brazilian collections house microorganisms obtained from different substrates in Brazilian territory. Even though the survey did not assess the number of isolates from each taxonomic group in the microbial collections or the quality and preservation status of these isolates, it is still reasonable to expect that bacteria and fungi are likely the most preserved microbial taxonomic groups in Brazil. These data illustrate the importance of culture collections in preserving Brazilian biodiversity.

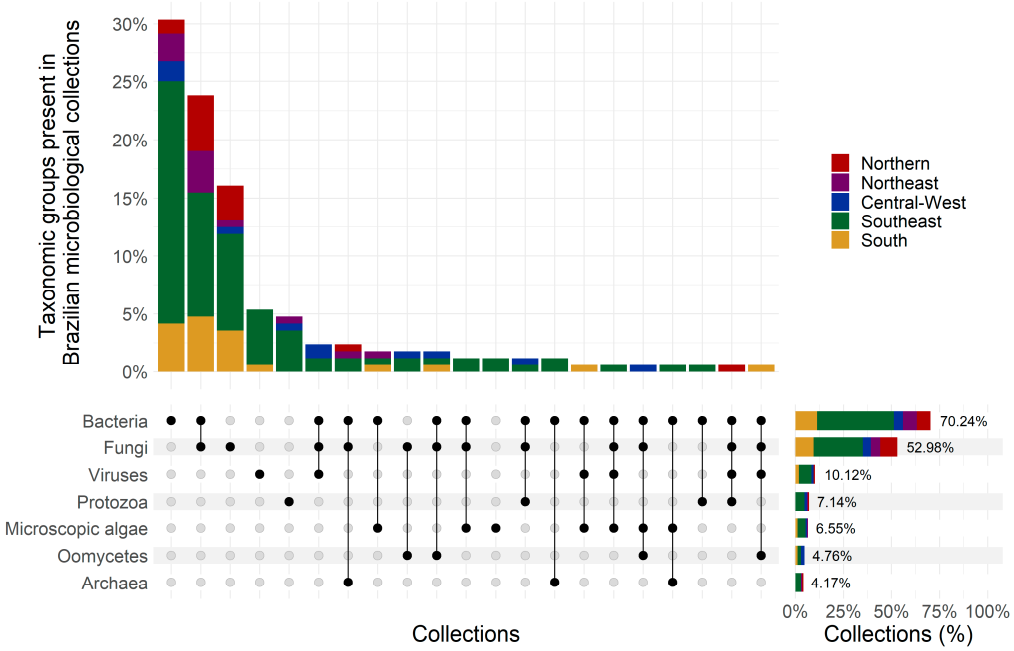

Figure 2. Brazilian microbiological collections predominantly safeguard bacteria and fungi. UpSet plot illustrating the distribution of taxonomic groups preserved in microbial collections across Brazilian regions. Black circles and vertical lines represent the intersections between taxonomic groups corresponding to each vertical bar.

3.3. One of the Most Diverse Biomes in Brazil Is Poorly Conserved Ex Situ

There is an evident heterogeneity between the biomes represented in Brazilian microbial collections (Figure 3). Most of the collections house microbial isolates obtained from the Atlantic Forest (40.48%), Amazon (35.12%), Cerrado (savannah) (33.33%), and Caatinga (26,19%); the Pantanal and Pampas biomes are poorly represented within the various microbial collections around Brazil (present in only 13.11% and 11.31% of the collections, respectively). This is surprising since the Pantanal is one of the world's most highly diverse biomes. Although plants and animals in the Pantanal are extensively studied, there are few studies on microorganisms, which is reflected in the low representation of this biome among the microbiological collections.

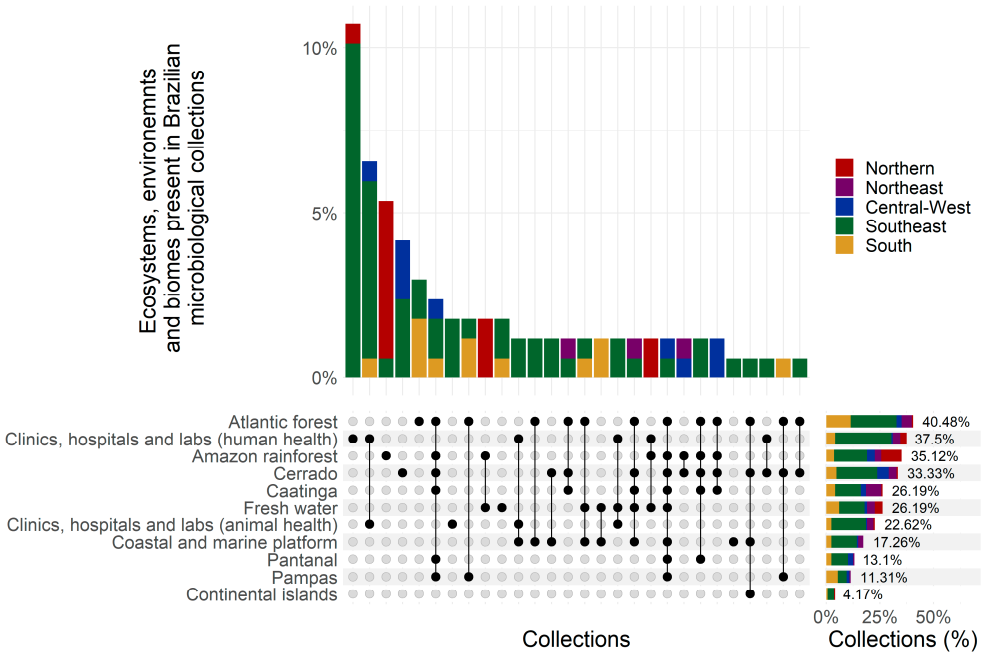

Figure 3. Brazilian microbiological collections predominantly safeguard microorganisms from the Atlantic Forest, Amazon rainforest, Cerrado, and Caatinga biomes. UpSet plot showcasing the distribution of represented ecosystems, biomes, and environments in Brazilian microbial collections across different regions. Black circles and vertical lines represent the intersections between ecosystems, environments and/or biomes corresponding to each vertical bar.

3.4. Brazilian Collections Are a Source of Microorganisms of Great Interest in Different Areas

A representative number of Brazilian collections harbor microorganisms with potential for application in several areas, being of interest in biotechnology, agriculture, and the pharmaceutical industry, in addition to their importance in clinical and veterinary medicine (Figure 4). A smaller number of collections are dedicated to preserving microorganisms of food interest (32.74%), demonstrating that there are still gaps to be filled.

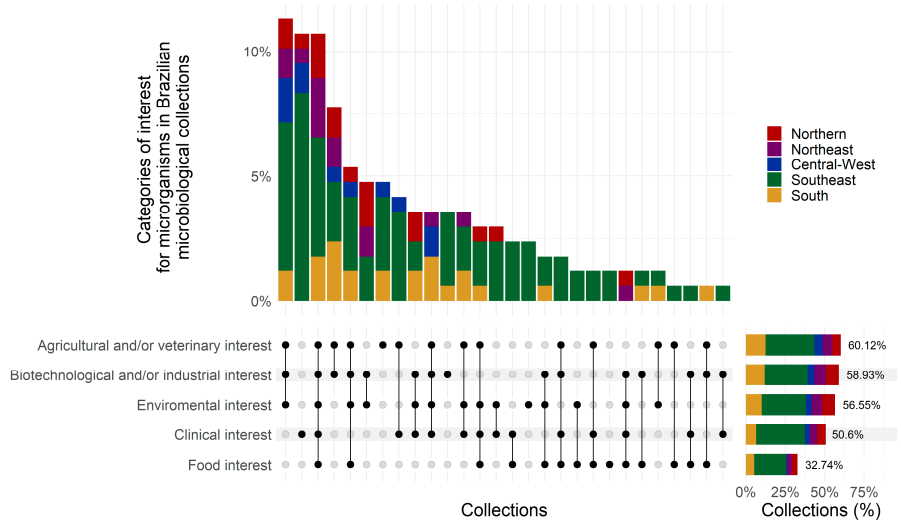

Figure 4. Brazilian microbial collections host diverse microorganisms of interest across various domains. UpSet plot illustrating the categories of interest for microorganisms in Brazilian microbial collections, highlighting their regional distribution. Black circles and vertical lines represent the intersections between categories of interest corresponding to each vertical bar.

Furthermore, we observed that several collections preserve clinical microorganisms (human and animal pathogens), suggesting that these groups also have great representativeness (Figure 4). The emergence and dissemination of multidrug-resistant pathogens, the introduction of new pathogens in Brazil (such as SARS-CoV-2, H5N1, H1N1, monkeypox, Zika virus, Chikungunya virus), and the emergence of new zoonoses highlight the importance of preserving these microorganisms for epidemiological studies and development of vaccines and new drugs.

3.5. Size of Brazilian Microbiological Collections and Methods of Preservation Applied

According to our study, 58.34% of Brazilian microbial collections can be considered small collections (up to 1000 isolates), 29.76% contain between 1000 and 5000 isolates, 9.5% are large collections (having between 5 and 50 thousand isolates), and one collection from the Southeast region (0.6%) contains more than 50,000 isolates (Figure 5). This last one is considered a very large microbial collection, larger than most collections in developed countries, according to Ryan and collaborators [5].

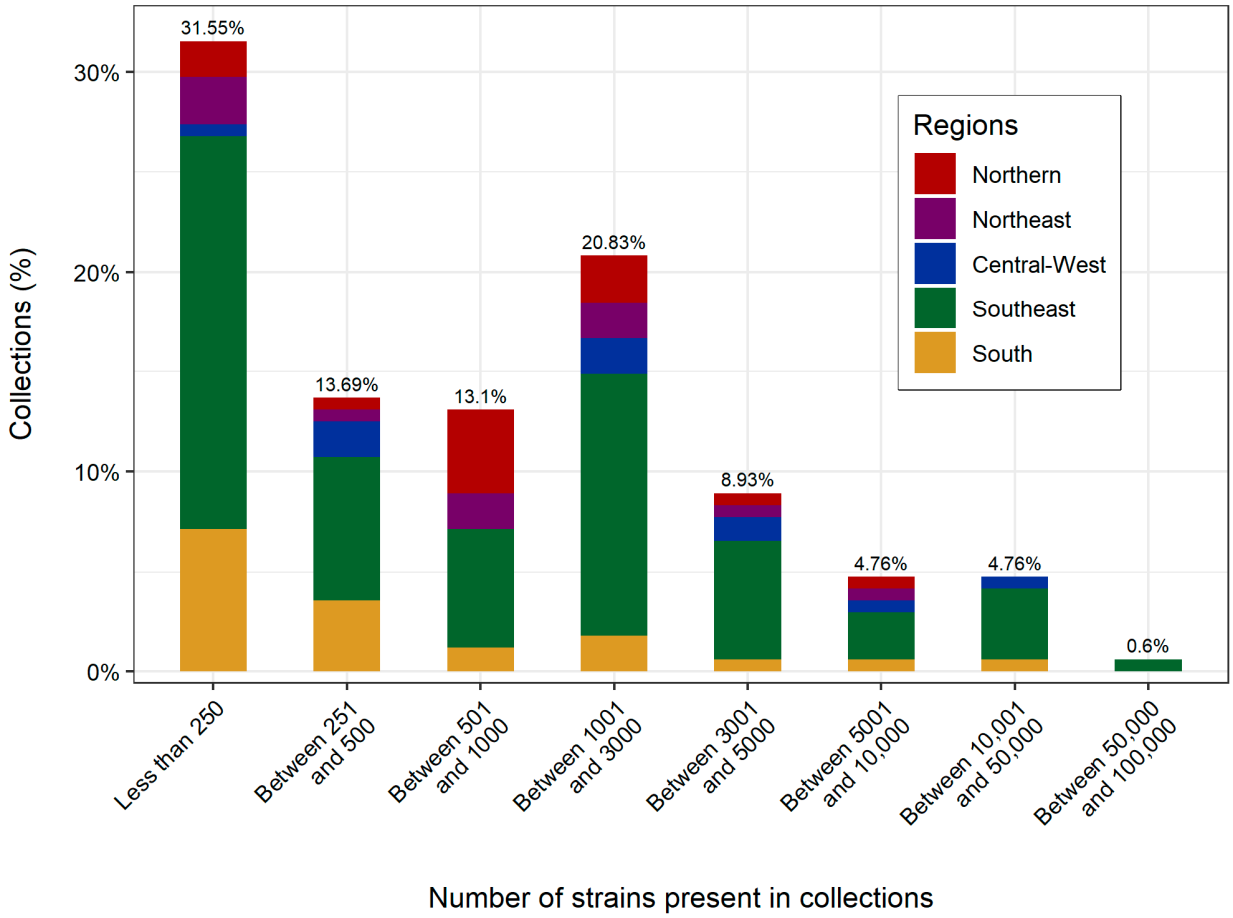

Figure 5. Brazilian microbial collections predominantly feature collections of small-to-moderate sizes. Stacked bar plots presenting the distribution of preserved strains in Brazilian microbial collections across different regions.

Regarding preservation, 66.67% of Brazilian microbiological collections employ cryopreservation at −80 °C as one of the preservation methods (Figure 6). However, several collections still preserve strains (including type strains) using classical methods, such as serial subculture (45.2%) and storage in oil (26.8%) or water (Castellani's method) (33.9%) [6]. One of the reasons is probably the limitation of financial resources to acquire equipment for cryopreservation.

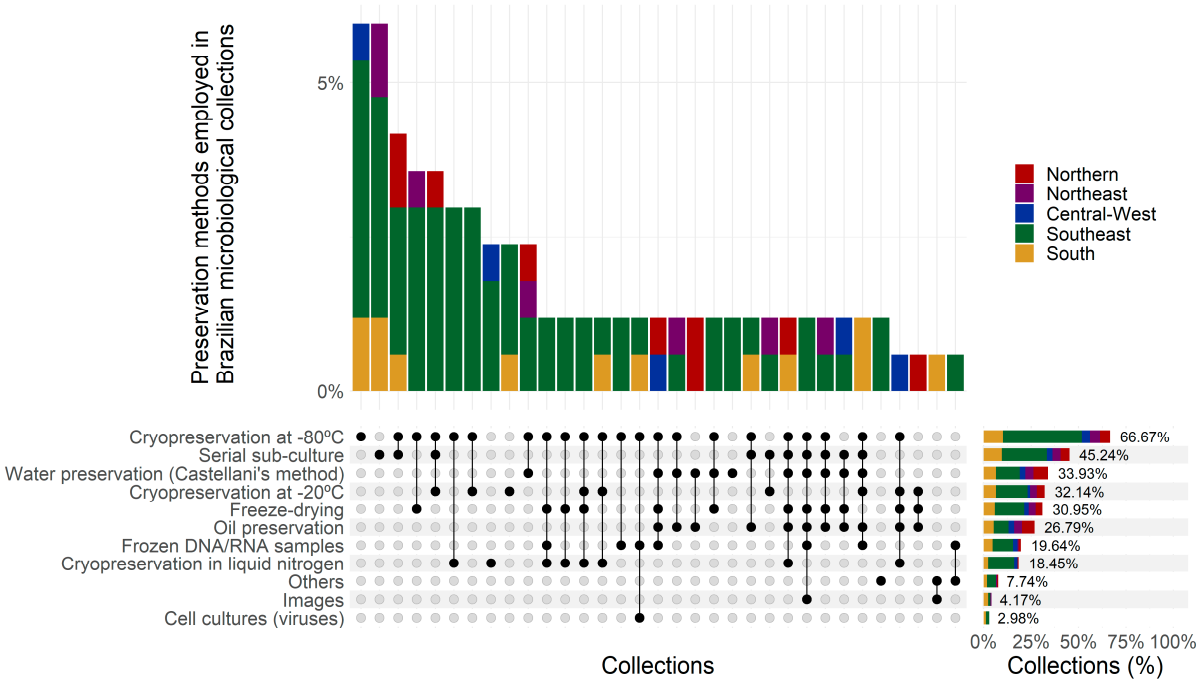

Figure 6. In addition to cryopreservation, numerous Brazilian microbial collections continue to utilize classical preservation methods for their strains. UpSet plot illustrating the distribution of preservation methods employed in Brazilian microbial collections across different regions. Black circles and vertical lines represent the intersections between preservation methods corresponding to each vertical bar.

However, if, on the one hand, these basic methods are low-cost and require only basic infrastructure, on the other hand, the main objective of preservation is to maintain purity and viability and avoid the selection of mutants [7]. Therefore, classical methods are not the best suited to meet these requirements, and there is a clear need to invest in long-term preservation methods such as freeze-drying and cryopreservation in ultra freezers and liquid nitrogen tanks [5].

In the routine maintenance of microbial collections, the quality and constant availability of reagents and basic supplies are of great importance, especially in maintaining the microorganisms in at least two different conditions [8]. However, there is a need for large financial investment for such maintenance, and unfortunately, such investment is limited for most Brazilian microbial collections, as will be shown below.

3.6. Despite Their Great Importance, Brazilian Microbial Collections Lack Infrastructure and Financial and Human Resources

Most microbiological collections in Brazil have serious problems with infrastructure, financial resources, and human resources. Regarding infrastructure, a considerable part of the collections (35.12%) are in a critical scenario, in which there is no preparation room, with the reception and expedition of microorganisms taking place in the same room in which the microorganisms are preserved (Figure 7).

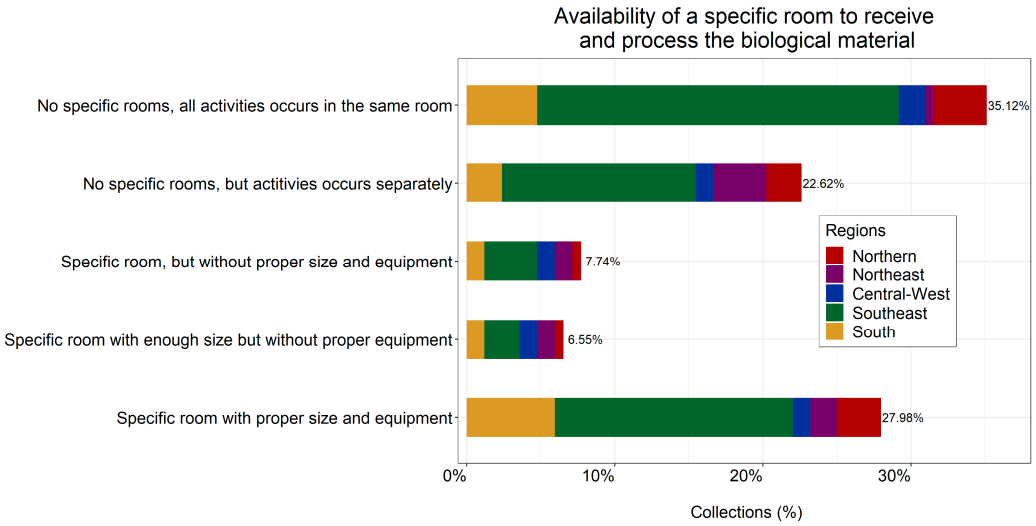

Figure 7. Most Brazilian microbial collections lack dedicated facilities for receiving and processing biological materials. Stacked bar plots showcasing the availability status of a designated rooms for receiving and processing biological materials in Brazilian microbial collections.

The lack of infrastructure is a critical issue for microbiological collections in Brazil, as 36.31% do not have a specific room to maintain the collection (Figure 8). But the scenario is even more worrying in some regions of Brazil, such as the North (53%) and Southeast (41%). Furthermore, for 23.2% of the Brazilian microbiological collections, even though they have a specific room to maintain the collection, the room is not large enough (Figure 8).

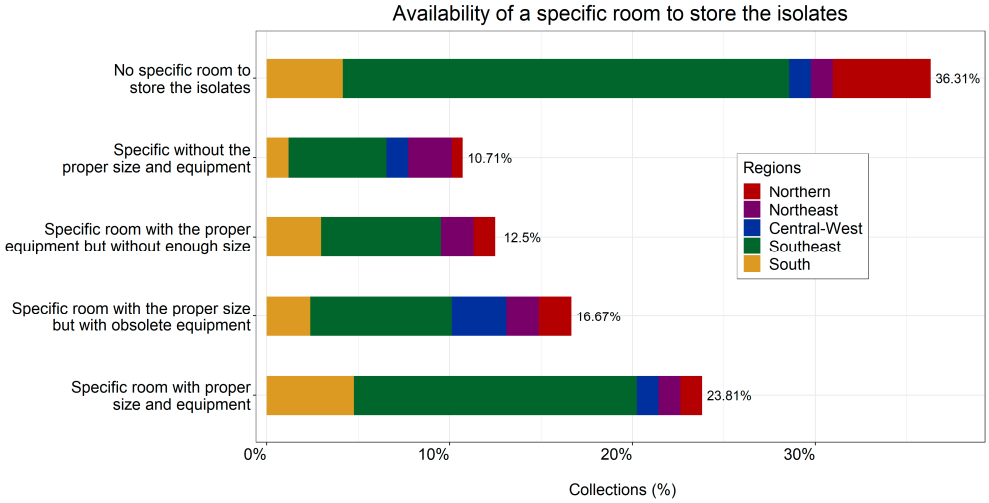

Figure 8. Most Brazilian microbial collections lack a dedicated room to store and maintain isolates. Stacked bar plots showcasing the availability status of a designated room to maintain isolates in Brazilian microbial collections.

In addition, there is also a concern regarding the security of the collection due to biosecurity issues [8]. Only 1.19% of the microbiological collections in Brazil have a complete security system specific to the collection, including security cameras and an entry and exit control system (Figure 9a). This figure also shows that 17.86% of the collections have no security system, neither for the institution nor for the collection. In addition, very few microbiological collections (1.19%) have a complete, exclusive, and adequately maintained fire protection system, including extinguishers, fire doors, sprinklers, alarms, smoke detectors, and a fire brigade (Figure 9b). Logically, in most institutions that house microbiological collections, there is a fire protection system in general (77.38%), but not

one exclusive to the collection. The positive point is that most microbial collections have adequate electrical installations (72.02%), which minimizes the risk of fires and the risks of freezers and ultra freezers not working, resulting in the loss of microorganism viability (Figure 9c).

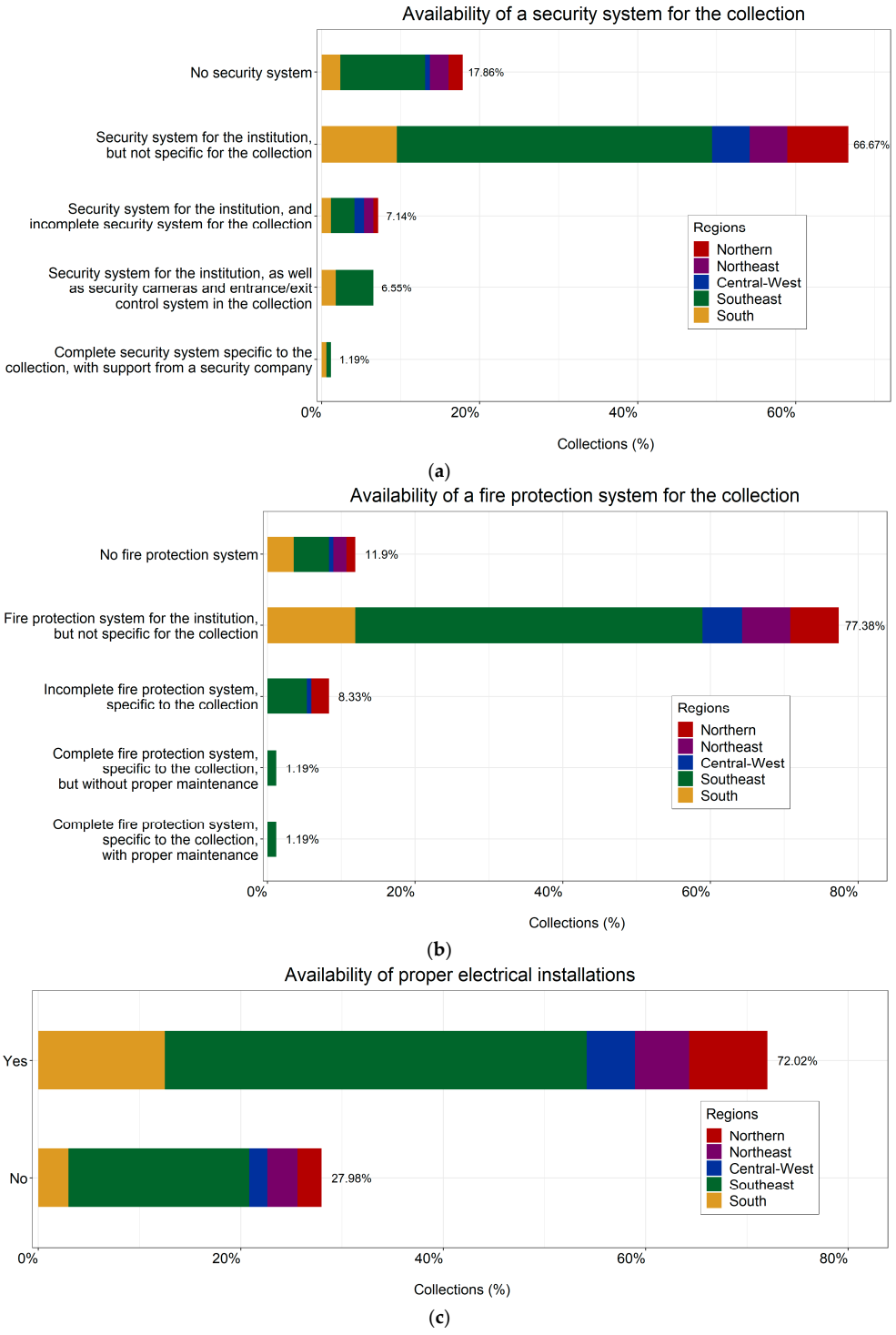

Figure 9. Brazilian microbial collections face infrastructure limitations across several aspects. Stacked bar plots illustrating the regional distribution of the availability status of security systems (**a**), fire protection systems (**b**), and adequate electrical installations (**c**) within the microbial collections.

As previously mentioned, unfortunately, the lack of funding is another fragile aspect of the current situation of microbial collections in Brazil, since most of them do not have sufficient financial resources to maintain their collections (Figure 10). These data reveal the risk to which collections are subjected since the lack of reagents, basic supplies, and cryopreservation equipment can compromise the quality, purity, and viability of the preserved material.

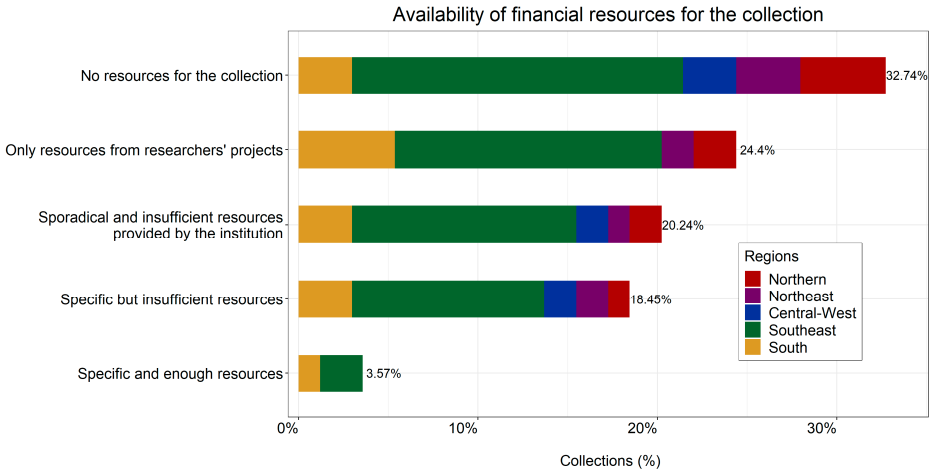

Figure 10. Brazilian microbial collections lack financial resources. Stacked bar plots showcasing the availability status of financial resources for the Brazilian microbial collections across different regions.

Another fragile area increasing these problems is the low number of professionals working in Brazilian microbiological collections (Figure 11). We analyzed four categories of professional attributions, and 54.76% of collections do not have professionals hired for the organization and maintenance of the collection. In some cases, these attributions are performed by volunteers or students with scholarships. These percentages are even higher for collection management (57.14%), data digitization (75%), or information technology (IT) support (86.31%).

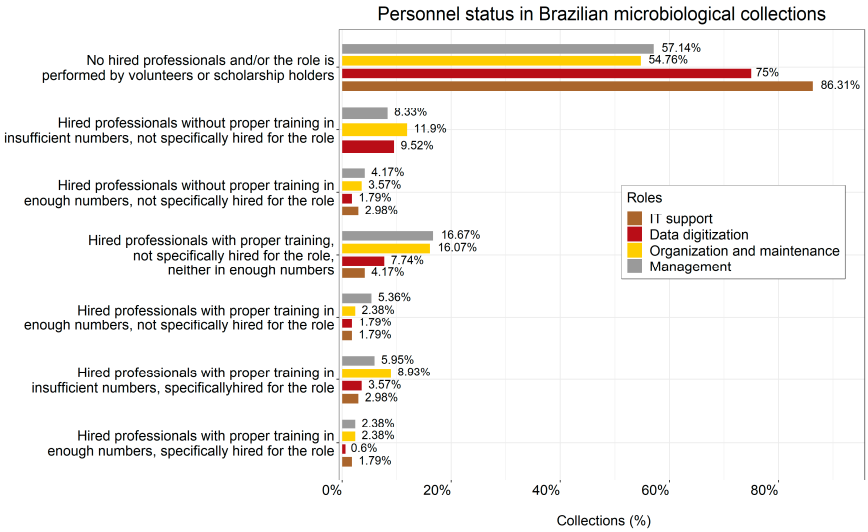

Figure 11. Insufficient dedicated professionals are associated with Brazilian microbial collections. Grouped bar plots showing the personnel status in Brazilian microbial collections in roles such as IT support (brown), data digitization (red), organization and maintenance (yellow), and management (gray).

3.7. Brazilian Microbiological Collections and Recognition by Institutions

A considerable part of Brazilian microbiological collections (58.33%) were founded between 11 and 50 years ago (Figure 12), having survived for an extended period and safeguarding material of great importance, as has been discussed so far. However, only 51.5% of collections have formal recognition from their institutions, with internal regulation and a specific policy for biological collections (Figure 13). Among these, approximately 56.9% are concentrated in the Southeast region. Therefore, the scenario in other regions of the country is even more critical.

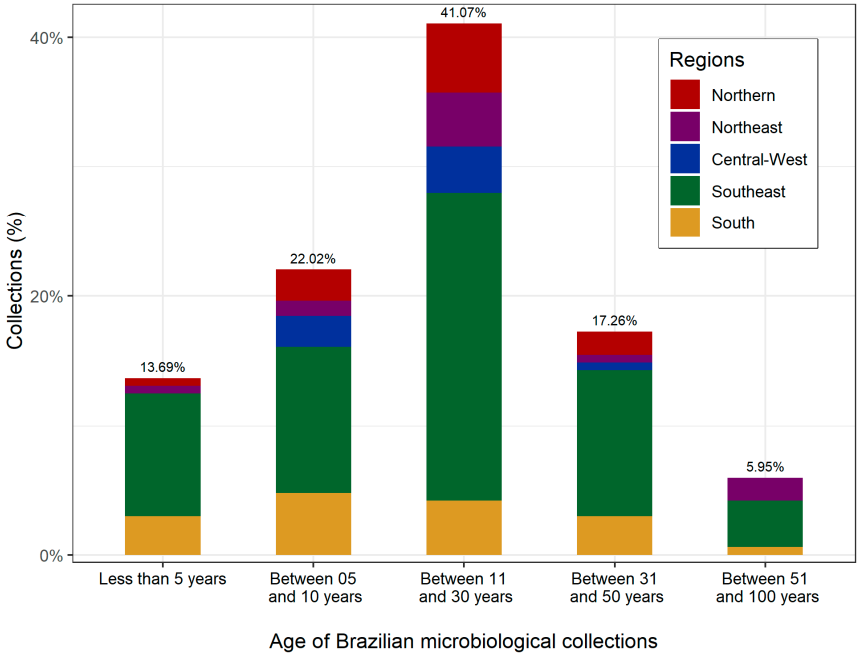

Figure 12. Most Brazilian microbial collections have been established for 11 to 30 years. Stacked bar plots showcasing the age distribution of Brazilian microbial collections since their foundation, organized by region.

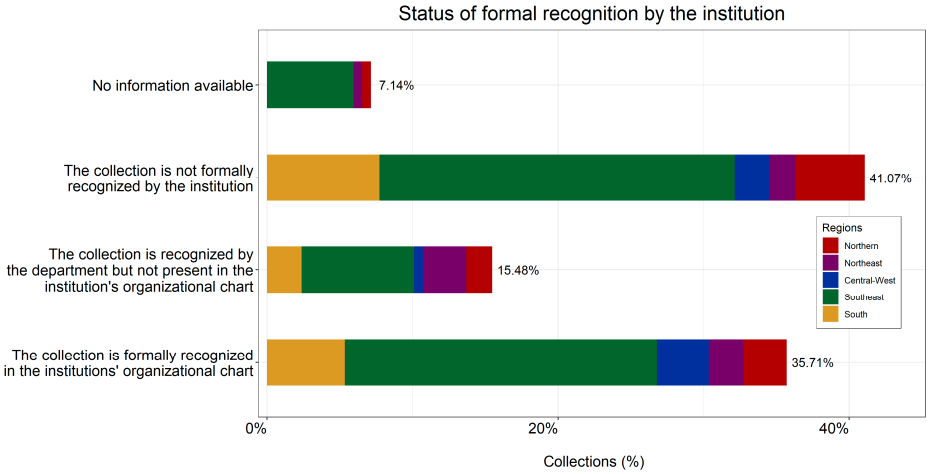

Figure 13. Many Brazilian microbial collections lack formal recognition from their respective institutions. Stacked bar plots showcasing the regional distribution of formal recognition status for these microbial collections.

Another essential piece of data to be analyzed is the official position of curator in the institutions' organizational chart. In 66.07% of the microbiological collections in this survey,

there is no curator position provided by their institutions (Figure 14). In the Southeast region, where the most significant number of collections are found, this percentage increases to 71.7%. These numbers are worrying, as they demonstrate that most collections rely on the commitment and dedication of researchers who act as curators voluntarily. Without the institution's formalization of curatorial activity, the collection risks disappearing when the researcher retires. The role of the curator is essential and must be a minimum criterion in the management, organization, and institutionalization of biological collections [9].

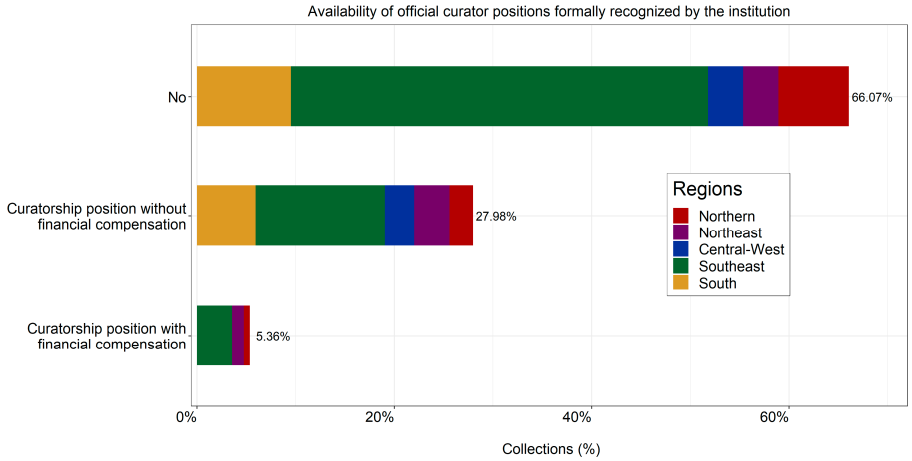

Figure 14. Most Brazilian microbial collections face a shortage of curatorship positions that come with formal recognition and financial compensation. Stacked bar plots displaying the status of curatorship positions in microbial collections across different regions.

In addition to the valuable material preserved in collections, microbial collections provide essential services that support research, technological development, and innovation [10]. Around 97% of the collections offer specialized services such as deposit and supply of strains, taxonomic identification, consultancy, courses and training, and others, and a significant proportion of these collections provide more than one kind of service (Figure 15). Although, they provide these services, they are not formally classified as service collections, which could change with institutional recognition.

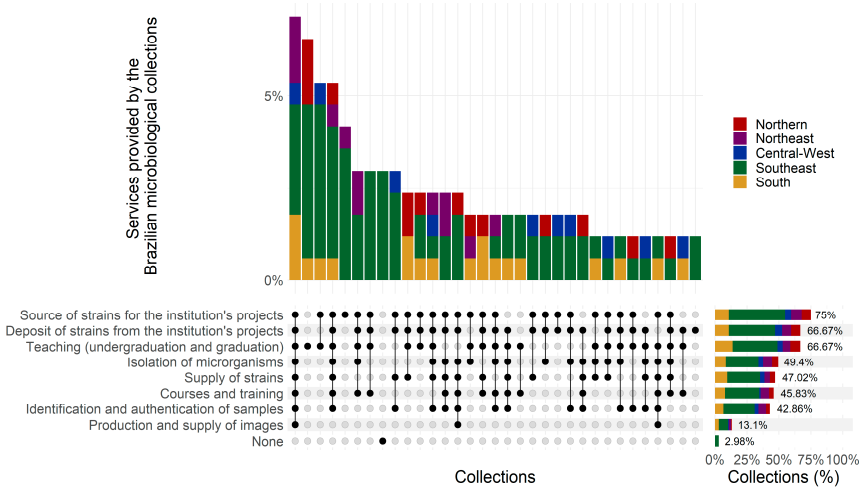

Figure 15. Brazilian microbial collections offer a diverse range of services catering to both public and private institutions. UpSet plot illustrating the regional distribution of services offered by Brazilian microbial collections. Black circles and vertical lines represent the intersections between services corresponding to each vertical bar.

4. Discussion

When discussing microbial collections, it is essential to adhere to certain minimum criteria: (1) the ability to conduct activities such as preservation, deposits, distribution, and taxonomic identification of microorganisms, along with scientific consultancy and training; (2) the presence of a curator; (3) the implementation of registration procedures and maintenance of documentation; and (4) possession of at least minimal human resources and infrastructure [9]. Achieving these criteria requires an urgent institutionalization of microbial collections and a commitment from their respective institutions to ensure their sustained maintenance. Such recognition is crucial for establishing minimum standards and regulations, thereby enforcing the quality of services. Consequently, this commitment ensures the provision of high-quality microbial resources, contributing to the reproducibility of research and, consequently, enhancing the credibility of science [11].

Considering the importance of microorganisms in promoting plant growth, in the cycling of compounds in nature, and in several other applications in the industry, agriculture, and health sectors, it is urgent to implement strategies aimed at the study, conservation, and prospecting of microorganisms distributed across all Brazilian biomes. Special emphasis is required for biomes underrepresented in Brazilian collections, notably the Pantanal. The significant diversity of plants and animals in the Pantanal biome suggests a rich microbial presence across different substrates, as suggested by several authors [12–15]. The data presented herein highlight the importance of comprehensive surveys to guide public strategies aimed at conserving the Brazilian microbiota in regions where assessment remains limited.

Unfortunately, a concerning aspect of the current situation in Brazil is the financial fragility faced by most microbiological collections, as illustrated in Figure 10. These data reveal the potential risk that collections are experiencing, given the lack of reagents, other basic supplies, and cryopreservation equipment. Such shortages may compromise the quality, purity, and viability of the preserved materials. To address this challenge, Brazilian microbial collections could benefit from the establishment of sustainability plans, incorporating external fundraising, inter alia, offering services and strains for sale. However, the shared responsibility of the public institutions which house the majority of these collections must be emphasized, as well as the government's obligation to provide financial support to ensure the sustainability of these collections.

In this framework, the Brazilian Access and Benefit Sharing legislation (Law 13,123/2015 [16]) presents a valuable opportunity for financing activities of Brazilian biological collections through the National Benefit Sharing Fund (FNRB). According to Decree 8772 of 2016 [17], which complements this law, Article 100 stipulates that the National Benefit Sharing Program is responsible for promoting various objectives, including I—the conservation of biological diversity; and II—the recovery, creation, and maintenance of ex situ collections of genetic heritage samples. The decree specifies that resources originating from the economic exploitation of finished products and reproductive material obtained through access to the Genetic Heritage from ex situ collections accredited at SisGen will be made accessible through the National Benefit Sharing Fund—FNRB [18]. Consequently, the Brazilian Access and Benefit Sharing legislation offer an opportunity to secure funding for activities and sustain Brazilian biological collections through the FNRB. However, to utilize resources from the Fund, collections must undergo accreditation via the Brazilian online system for access and benefit sharing framework, SisGen; a process coordinated by the legal representative of each institution.

On the other hand, regrettably, the implementation of Law 13,123/2015 has resulted in adverse effects on microbiological collections, particularly regarding the description of new species. According to this law, foreign researchers seeking access to native Brazilian biodiversity can only do so if they are affiliated with public or private Brazilian scientific and technological research institutions, and, therefore, the responsibility for registering the activity in SisGen (https://sisgen.gov.br/paginas/login.aspx) falls on these institutions. This requirement is also applicable when utilizing any Brazilian genetic resource deposited in ex situ collections. This prerequisite has led to a reluctance of international microbio-

logical collections to accept deposits from Brazilian microorganisms in recent years. In addition, it has also created challenges for the characterization of new species of bacteria originated from Brazil, as depositing type strains in at least two different culture collections is mandatory. According to da Silva and collaborators [18], the association requirement outlined in Brazilian Law 13,123/2015 contradicts the principle of "unrestricted distribution" envisaged under Rule 30 of the International Code of Nomenclature of Prokaryotes (ICNP), significantly impacting studies involving Brazilian biodiversity. Despite ongoing efforts to address these issues, many challenges persist, indicating that there is still much progress to be made.

Consolidating this information and recognizing the crucial role that ex situ collections play in preserving and conserving biodiversity, it becomes evident that these initiatives demand substantial financial resources, requiring Brazilian microbiological culture collections to actively seek investments and recognition.

5. Conclusions

Brazilian ex situ collections preserve microorganisms of high importance and with potential for application in different sectors. However, despite all these economic and biotechnological potentials, our data analysis shows severe limitations and fragilities, especially regarding physical infrastructure and human resources, and raises alerts about the risk that Brazilian collections are subjected to.

In conclusion, we recommend that institutions commit to the maintenance and preservation of their microbiological collections and become responsible for their management and organization. We also recommend that the Brazilian government invest more in taxonomy, data handling, bioinformatics, and culture collection management and training. These supports and investments will strengthen the preservation of Brazilian biodiversity in different biomes. They will support the bioeconomy, allowing important microorganisms to be applied in several areas, including human health research, antimicrobials, environmental protection, bioinoculants, and biocontrol agents for sustainable agriculture and even for animal and human food, as well as for taxonomic purposes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16020116/s1, Table S1: Applied survey containing questions and options provided to those responsible for microbiological collections.

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Data Availability Statement: We hereby declare that it is not possible for us to make available all the data resulting from the diagnosis of biological collections since they are still being used for other analyses and must be preserved.

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