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Original Article

The *Yersinia pestis* Collection (Fiocruz/CYP): history, challenges, and contributions to epidemiological surveillance in the Global South

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ABSTRACT

Microbiological collections are essential for scientific research and public health, serving as critical repositories of biological diversity and resources for taxonomic, epidemiological, and genomic studies. In Brazil, the *Yersinia pestis* collection at the Oswaldo Cruz Foundation (Fiocruz/CYP) represents a unique and invaluable resource, being the largest repository of its kind in South America. Established in 1966, Fiocruz/CYP has grown to include 917 Brazilian strains, supporting research on phylogenetics, molecular epidemiology, and antimicrobial resistance. The collection also provides essential scientific services, including molecular characterization, taxonomic identification, training in biosafety protocols, as well as robust preservation strategies and optimized culture media to ensure strain viability and genetic stability. Genomic and phylogenetic analyses of Fiocruz/CYP strains reveal significant genetic diversity, evolutionary patterns, and potential virulence attenuation, offering insights into the bacterium's adaptation and transmission dynamics. Integrated with the Brazilian National Plague Reference Service, the collection plays a pivotal role in epidemiological surveillance, outbreak response, and public health interventions. Here, we provide a detailed analysis of Fiocruz/CYP, exploring its history, composition, and significance while highlighting its contributions to research, epidemiological surveillance, and public health, as well as discussing the challenges and future perspectives for its preservation and utilization.

Keywords: *Yersinia pestis*; microbiological collections; epidemiological surveillance

INTRODUCTION

Microbiological collections play a pivotal role in scientific research, public health, and biosafety, functioning as essential repositories of biological diversity and providing critical resources for taxonomic, epidemiological, and genomic investigations (Meineke *et al.* 2018, Silva *et al.* 2020). Among pathogenic microorganisms of historical and medical relevance, *Yersinia pestis* is particularly notable as the causative agent of plague, a disease that has precipitated multiple pandemics throughout human history (Barbieri *et al.* 2020, Glatter and Finkelman 2021). The bacterium's persistence in enzootic reservoirs, its potential for re-emergence, and its classification as a Category A bioterrorism agent underscore the imperative for maintaining well-documented and rigorously preserved reference strains (Glatter and Finkelman 2021).

In Brazil, the *Y. pestis* strain collection maintained at the Oswaldo Cruz Foundation (Fiocruz/CYP, http://cyp.fiocruz.br/) constitutes a unique and invaluable resource for scientific research and public health surveillance (da Rocha *et al.* 2010). As the sole repository dedicated to *Y. pestis* in the country and the largest in South America, it encompasses strains acquired over decades of plague surveillance and epidemiological investigations (da Rocha *et al.* 2010). Beyond its historical significance, the strains in the collection represent Brazil's biodiversity and genetic heritage, serving as a valuable resource for comparative studies. Fiocruz/CYP is associated with the Brazilian National Plague Reference Service (*Serviço Nacional de Referência em Peste*, SRP) and linked to the speciesLink network (https://specieslink.net/col/Fiocruz-CYP/#:~:text=A%20Cole%C3%A7%C3%A3o%20

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de%20Yersinia%20pestis,1966%20a%20novembro%20de%201974), which integrates primary data from Brazilian biological collections. Additionally, the collection is registered with the World Federation for Culture Collections (WFCC) under the code WDCM 1040.

Fiocruz/CYP plays a critical role in advancing both national and international research initiatives, serving as a reference for phylogenetic analyses, molecular studies, and investigations into antimicrobial resistance (da Rocha *et al.* 2010, Pitta *et al.* 2023, Bezerra *et al.* 2024). Additionally, it contributes to the enhancement of diagnostic capabilities, the strengthening of laboratory infrastructure, and the formulation of strategies for plague surveillance and control.

Despite significant progress in plague control and a dramatic reduction in human cases in Brazil, *Y. pestis* continues to circulate in rodent reservoirs and their associated ectoparasites, representing a persistent public health concern (Bezerra *et al.* 2024, da Rocha *et al.* 2025). The preservation and analysis of both historical and contemporary strains are crucial for elucidating the bacterium's evolution, pathogenic mechanisms, and ecological interactions. Furthermore, the Fiocruz/CYP collection aligns with international initiatives to enhance biosecurity protocols (Davis *et al.* 2016), ensuring that reference strains are maintained under stringent biosafety conditions while remaining accessible for scientific research and innovation.

This article provides a comprehensive overview of the history, composition, and significance of the Fiocruz/CYP *Yersinia pestis* collection, highlighting its contributions to research, surveillance, and public health, as well as discussing the challenges and future perspectives for its preservation and utilization. The analysis draws on the extensive archives of the SRP, integrating the practical expertise of the team with a thorough review of historical records, epidemiological studies, and scientific literature.

History and formation of the collection

Fiocruz/CYP was initiated in 1966 as part of the Pilot Plague Project (PPP) in the municipality of Exu, Pernambuco - Brazil, a collaborative initiative between the Brazilian government and the World Health Organization (WHO) (da Rocha *et al.* 2010). This extensive research programme was conducted in the Chapada do Araripe region, located in the state of Pernambuco, Brazil, from July 1966 to November 1974. During this period, 661 strains of *Y. pestis* were isolated from human cases, rodents, and fleas, marking the beginning of the collection (da Rocha *et al.* 2010).

Following the conclusion of the PPP in 1974, the collection was transferred to the Central Laboratory of Garanhuns, Pernambuco, which had been designated as the National Diagnostic Center for Plague (da Rocha *et al.* 2010). At this facility, the research activities initiated in Exu were continued, and the collection was expanded with additional strains obtained through ongoing surveillance and control efforts in plague-endemic regions. In 1982, the collection was further institutionalized through its relocation to the Aggeu Magalhães Institute (IAM) at the Oswaldo Cruz Foundation (FIOCRUZ) in Pernambuco. Over subsequent years, the Fiocruz/CYP collection experienced significant growth, both in the number of strains and in its geographical representation. This expansion continued until 1997, when the final specimens collected within Brazil were integrated into the collection (da Rocha *et al.* 2010).

In 2007, the collection was officially recognized by the Permanent Forum of Biological Collections of Fiocruz, which recommended its formal institutionalization. The collection was then designated as the *Yersinia pestis* Collection, adopting the acronym Fiocruz/CYP, thereby solidifying its status as a valuable resource for research, diagnostics, and reference purposes (da Rocha *et al.* 2010). Figure 1 illustrates the historical trajectory of Fiocruz/CYP.

Currently, the collection comprises 917 *Y. pestis* strains from Brazil. In addition, 15 international strains—isolated in the United States, Peru, Vietnam, Iran, Java, and Burma—are available for comparative studies. These international specimens were provided by esteemed institutions such as the Pasteur Institute of Paris (IPP, France), the National Institute of Health (INS, Peru), the Centers for Disease Control and Prevention (CDC, Fort Collins, CO, USA), and Cleveland University (OH, USA).

A significant portion of the Brazilian strains (770, 84.5%) originates from the Chapada do Araripe, a recognized plague focus spanning the states of Pernambuco (PE), Ceará (CE), and Piauí (PI), which has historically been the primary hotspot for *Y. pestis* isolation in the country. Other *Y. pestis* strains were isolated from Serra da Ibiapaba (49, 5.4%), Planalto da Borborema (47, 5.2%), Serra de Triunfo (30, 3.3%), Serra de Baturité (8, 0.88%), Planalto Oriental da Bahia (4, 0.44%), and Vale do Jequitinhonha (2, 0.22%). Further details on the geographical distribution, period of isolation, and source of the Fiocruz/CYP isolates are presented in Figure 2.

Maintenance and preservation strategies

The long-term preservation of the strains is essential to maintaining genetic stability, viability, and reproducibility for future research. Currently, two primary storage methods are employed at Fiocruz/CYP: high-layer peptone agar (HLPA) and cryopreservation with Luria–Bertani (LB) medium with 15% glycerol

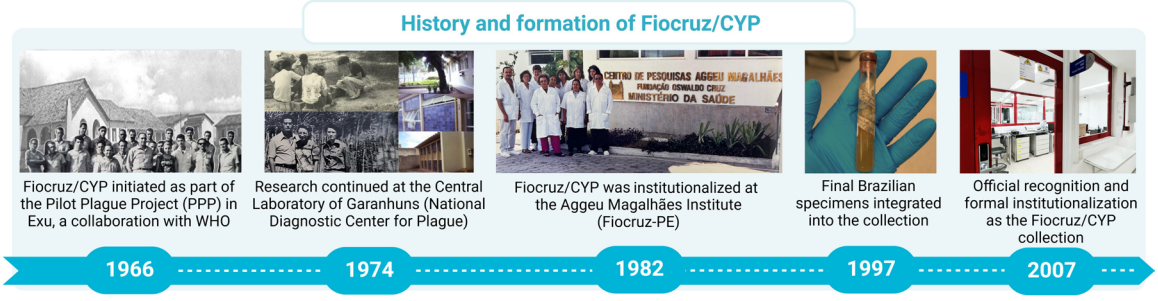

Figure 1. Historical timeline of the Fiocruz/CYP collection.

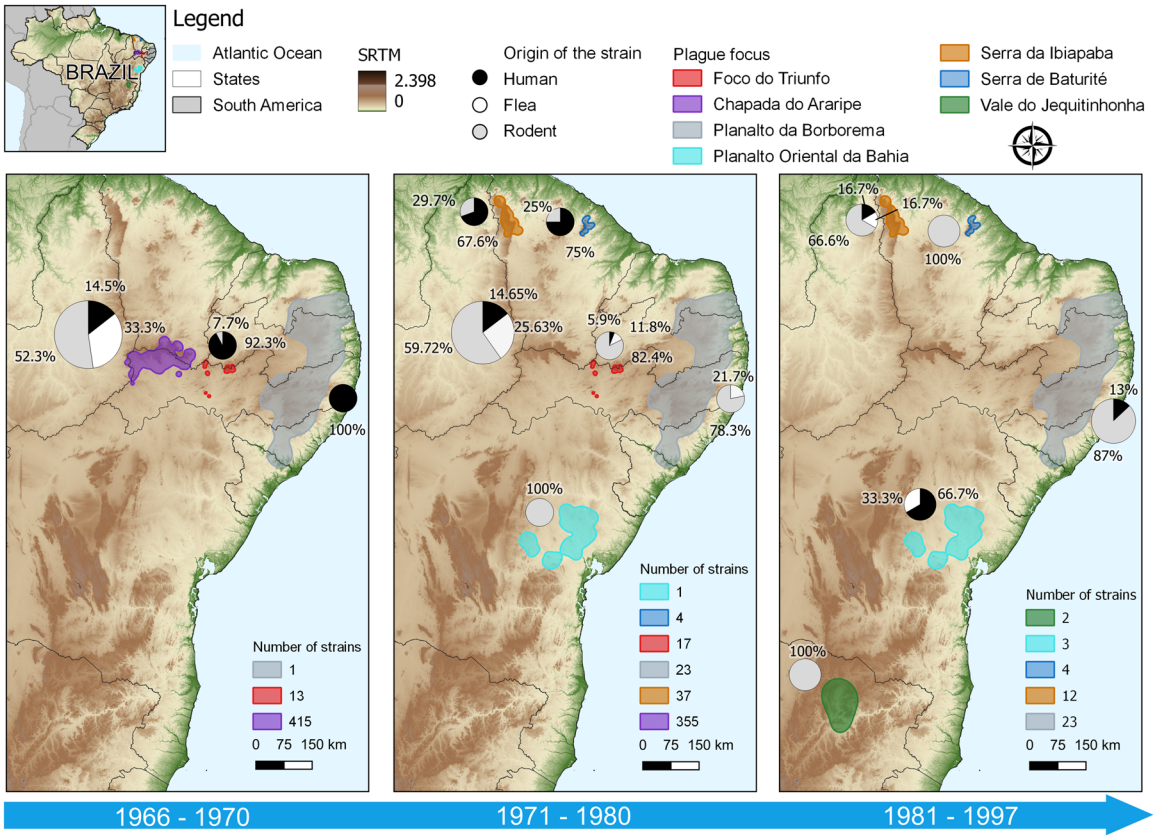

Figure 2. Spatial and temporal distribution of *Y. pestis* strains from the Fiocruz/CYP collection, showing plague foci (coloured regions), strain origins (human, flea, rodent), and topography (Shuttle Radar Topography Mission, SRTM, elevation data from OpenTopography, https://opentopography.org/). Percentages indicate strain proportions per region; numbers represent total sampled strains. Scale bar: 75–150 km. Time periods: 1966–1997.

at −80°C. HLPA provides a stable environment for short-term storage under refrigeration (4 to 8°C), preserving bacterial viability and phenotypic characteristics (Leal *et al.* 2016). However, extended storage under these conditions has been associated with reduced recovery rates and an increased risk of contamination (Leal *et al.* 2016). A preliminary study identified low recovery rates from peptone agar tubes, often compromised by fungal or bacterial contamination (Leal *et al.* 2016). In response, a selective LB-based liquid medium, designated CYP broth, was developed to enhance the recovery of Fiocruz/CYP strains from long-term storage and field-collected samples (Rocha *et al.* 2023). This medium has demonstrated increased efficiency in reviving stored cultures while simultaneously mitigating contamination risks, marking a significant advancement in bacterial preservation methodologies.

In contrast to HLPA preservation, cryopreservation in LB with glycerol is widely used for long-term storage, effectively reducing metabolic activity and genetic drift while ensuring higher viability upon recovery (Rocha *et al.* 2024). Despite its benefits, cryopreservation presents significant limitations, primarily due to the requirement of ultra-low-temperature freezers, which impose logistical and financial challenges, particularly in resource-limited settings (Rocha *et al.* 2024).

In addition to culture-based preservation, lyophilization has been explored as a complementary strategy for long-term storage of *Y. pestis* strains. A modified lyophilization protocol was developed to assess its effects on viability and stability of Fiocruz/CYP strains (Rocha *et al.* 2024), aiming to replace or complement existing methodologies. Notably, the refined protocol eliminated the need for a sterile lyophilizer, streamlining the process while maintaining high recovery rates. By integrating these novel approaches, the Fiocruz/CYP collection has enhanced its preservation protocols, ensuring the long-term maintenance of *Y. pestis* strains with minimal genetic drift and contamination risks. Figure 3 summarizes the main activities and studies conducted by Fiocruz/CYP.

Scientific services

Fiocruz/CYP provides a comprehensive range of scientific services that support research, diagnostics, and epidemiological surveillance. Given that *Y. pestis* is classified as a Category A bioterrorism agent (Glatter and Finkelman 2021), the distribution of viable strains is strictly regulated and only purified DNA is made available for research purposes. These samples are provided exclusively to recognized institutions upon formal request, ensuring full adherence to biosafety regulations and international biosecurity standards.

In addition to providing DNA samples, the collection offers comprehensive taxonomic identification of *Y. pestis* isolates through bacteriological culture characterization, specific bacteriophage susceptibility assays, and biochemical tests for biovar classification, along with antimicrobial susceptibility testing.

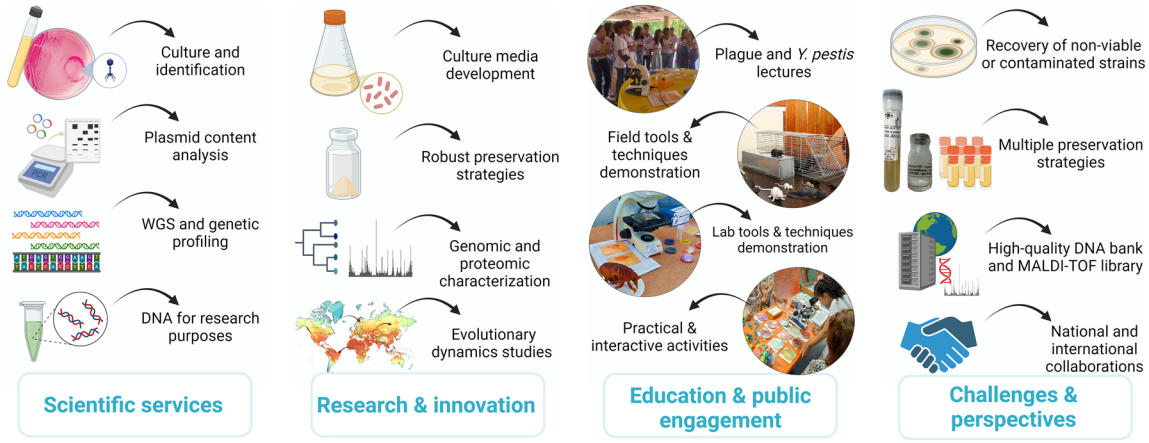

Figure 3. Key activities and contributions of Fiocruz/CYP: maintenance, services, and research. WGS: Whole-genome sequencing. MALDI-TOF: Matrix-Assisted Laser Desorption/Ionization Time-of-Flight.

Molecular characterization represents another key service provided by Fiocruz/CYP, employing advanced methodologies such as plasmid content analysis, whole-genome sequencing, and ribosomal 16S subunit sequencing for precise identification and comprehensive genetic profiling of *Y. pestis* strains. The overview of these scientific services is illustrated in Figure 3. Beyond research and diagnostic services, the collection is also dedicated to training researchers and professionals in biosafety protocols, microbiological techniques, and molecular diagnostics, ensuring proficiency in the safe handling of *Y. pestis* and the application of advanced methodologies.

Research and innovation

The Fiocruz/CYP collection is an invaluable resource for various research initiatives and innovations. Numerous studies using these strains have significantly advanced the understanding of *Y. pestis* and its pathogenic mechanisms (Leal *et al.* 2016, Pitta *et al.* 2023, Rocha *et al.* 2023, 2024, Bezerra *et al.* 2024). Initial identification of the cultures was performed based on their characteristics on Blood Agar Base and MacConkey Agar, testing with the *Y. pestis*-specific bacteriophage, and biochemical assays for biovar determination. Over time, the strains in the collection have been extensively characterized through multiple molecular approaches, including plasmid content analysis, outer membrane protein profiling, Random Amplified Polymorphic DNA (RAPD), ribotyping-PCR, Multiple-Locus Variable-number Tandem Repeat Analysis (MLVA), Pulsed-Field Gel Electrophoresis (PFGE), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) locus analysis (Leal and Almeida 1999, Cavalcanti *et al.* 2002, Leal-Balbino *et al.* 2006, Oliveira *et al.* 2012, Barros *et al.* 2013, Pessoa-Junior *et al.* 2014). These methodologies have provided critical insights into the genetic diversity, epidemiological distribution, and evolutionary history of the bacterium.

In recent years, the development of optimized culture media for the isolation and growth of *Y. pestis* strains, along with advancements in lyophilization techniques, has significantly improved the viability and long-term preservation of the Fiocruz/CYP collection (Rocha *et al.* 2023, 2024). Current research efforts are focused on three key areas: the evolutionary dynamics of *Y. pestis* through georeferencing, comprehensive genomic and proteomic characterization, and the refinement of diagnostic and surveillance methodologies (Bezerra *et al.* 2022a, b, 2024, Pitta *et al.* 2023). Whole-genome sequencing is being employed to investigate genetic variations, adaptive mechanisms, and phylogenetic relationships among strains, providing crucial insights into the bacterium's evolutionary history (Pitta *et al.* 2023). Concurrently, proteomic analysis using MALDI-TOF mass spectrometry is enabling the identification of key protein markers, enhancing the accuracy of *Yersinia* species identification (Rocha *et al.* 2025). Additionally, georeferencing data are being systematically incorporated into the collection, allowing for precise tracking of strain origins and their spatial distribution (Bezerra *et al.* 2024). Figure 3 provides an overview of the research and innovation activities enabled by the collection.

Genomic and phylogenetic characteristics of Fiocruz/CYP Y. pestis strains

The *Y. pestis* strains within Fiocruz/CYP exhibit notable genomic and phylogenetic features that reflect their genetic diversity, evolutionary history, and potential virulence profiles. Genomic analyses of the collection (Bezerra *et al.* 2024) reveal a conserved core genome equivalent to 46% of the CO92 reference strain (GenBank accession no. GCA_000009065.1). Phylogenetic studies place Brazilian strains within the 1.ORI phylogenetic population, forming a monophyletic clade that is closely related to strains from South and North America, which, in turn, branch from strains originating in Yunnan and Southeast Asia (Bezerra *et al.* 2024).

Phylogenetic studies of the spatial and temporal distribution of *Y. pestis* lineages have demonstrated that strains from the same geographic plague foci cluster together, with Brazilian isolates forming a relatively homogeneous genetic group (Bezerra *et al.* 2024). Notably, strains from the Chapada do Araripe are characterized by a foundational ancestral lineage, which gave rise to transient genetic groups during outbreaks. For instance, one such group emerged in 1969, rapidly expanding before being replaced by the ancestral lineage. In contrast, another group that emerged during the 1974–1975 outbreak became the dominant lineage, coinciding with a significant increase in plague cases (Bezerra *et al.* 2024).

Pangenome analysis of the Fiocruz/CYP collection has identified a comprehensive array of virulence factors, such as *ail* (involved

in attachment and invasion), *astA* (enterotoxin), and genes related to chemotaxis (*cheB*, *cheD*, *cheR*, *cheW*, *cheY*, and *cheZ*). The strains of the collection also harbour genes encoding and regulating pesticin/yersiniabactin (*fyuA*, *irp1*, *ybtA*, *ybtE*, *ybtQ*, *ybtS*), components of the type III secretion system (*lcrE/yopN*, *lcrO/yscI*, *lcrQ/yscM*, *virG/yscW*, *ylpB/yscJ*, *yopB*, among others), low calcium response proteins (*lcrR* and *lcrV*), plasminogen activator (*pla*), adhesion factors (*psaA*), and genes encoding the YopE and YopH proteins, which are critical for virulence. The murine toxin gene (*ymt*) is also present in some strains (these data are reported here for the first time and are to be published elsewhere).

Additionally, genomic analysis reveals substantial variability in virulence factors among different genetic groups, with some groups exhibiting distinct patterns of gene absence that suggest potential attenuation of virulence over time. For instance, certain groups lack genes critical for biofilm formation, iron acquisition, and cell adhesion, all of which are essential for *Y. pestis* pathogenicity (Pitta *et al.* 2023). Notably, genes from the *caf* operon, responsible for the F1 antigen that inhibits phagocytosis, are absent in some strains, as are genes from the *psa* family, which play a role in pH6 antigen expression and virulence. Another significant observation is the absence of the *pld* gene (*Yersinia* murine toxin) in some strains, as well as the lack of genes associated with iron acquisition and transport in certain groups, further supporting the hypothesis of virulence attenuation over time (Pitta *et al.* 2023).

In addition to virulence factors, resistance genes such as *rosA* and *rosB*, associated with multi-drug efflux pumps, and CRP, a regulator of drug and biocide efflux, have been identified. Plasmid analysis revealed the presence of IncFII(Y)_1_ps, IncFIB(pHCM2)1_pHCM2, and ColRNAI_1 plasmids which may contribute to the genetic adaptability and virulence of the strains. Additionally, traces of bacteriophages from the Siphoviridae and Myoviridae families were identified, suggesting potential horizontal gene transfer events that could influence the evolution and pathogenicity of *Y. pestis* within the collection (to be published elsewhere).

Epidemiological and evolutionary patterns studies

The integration of genomic data of Fiocruz/CYP strains with epidemiological metadata reveals distinct evolutionary patterns associated with isolation years and outbreak events. Using a minimum spanning tree approach, primary clusters of strains were identified, with the largest cluster predominantly consisting of strains from the 1974–1975 outbreak (Pitta *et al.* 2023, Bezerra *et al.* 2024). This cluster is further subdivided into subclusters associated with specific outbreaks in regions such as the Chapada do Araripe and Serra de Triunfo.

Despite the observed genetic diversity, no clear patterns emerge between genomic profiles and collection locations, periods, or hosts. However, some Fiocruz/CYP *Y. pestis* groups exhibit high epidemiological diversity, while others exhibit a more distinct association with specific plague foci. The temporal and spatial dynamics of *Y. pestis* lineages reveal the replacement of previously dominant strains by new genetic groups, which coincided with significant plague outbreaks (Pitta *et al.* 2023, Bezerra *et al.* 2024).

Importance of Fiocruz/CYP for public health

Fiocruz/CYP plays a pivotal role in public health and biosafety, particularly through its integration with the SRP. As a reference centre for plague, the SRP relies on this collection to support epidemiological surveillance, outbreak response, and research on *Y. pestis*. The Collection provides essential genomic and phenotypic data that aid in identifying emerging strains, tracking transmission patterns, and elucidating the evolution of virulence factors. This information is indispensable for guiding public health interventions, such as targeted vaccination campaigns and vector control strategies, especially in regions where plague remains endemic.

Education, outreach, and public engagement

Fiocruz/CYP actively participates in scientific education, outreach, and public engagement initiatives. The collection develops interactive educational materials and games, which are featured in exhibitions designed for diverse audiences, employing creative and inclusive language. These initiatives aim to bridge the gap between the scientific community and society, fostering scientific literacy and encouraging the social appropriation of scientific knowledge, thereby expanding opportunities for inclusion. The exhibitions cover topics ranging from the historical context of plague in Brazil—highlighting data associated with the collection's archives—to the daily field activities of plague surveillance and the laboratory procedures involved in maintaining and preserving the collection's specimens.

Challenges and perspectives

The continuous expansion and maintenance of the Fiocruz/CYP collection face several challenges, particularly in ensuring the viability and long-term preservation of *Y. pestis* strains. One of the key ongoing efforts is the recovery of contaminated or non-viable strains, which requires specialized protocols to optimize growth conditions and restore lost isolates. Additionally, a major priority is the implementation of multiple preservation strategies for each *Y. pestis* strain, including high-layer agar, glycerol storage at −80°C, and lyophilization, to enhance stability and longevity.

Another critical objective is the establishment of a robust and standardized DNA bank composed of high-quality, lyophilized genomic material. This initiative aims to facilitate genetic and phylogenetic studies while ensuring long-term accessibility for research and diagnostic applications. Furthermore, efforts are underway to develop a comprehensive MALDI-TOF spectral library of *Y. pestis* strains within the collection. This resource will significantly enhance microbial identification capabilities and support proteomic analyses relevant to bacterial taxonomy, virulence factors, and antimicrobial resistance profiles.

Future perspectives for Fiocruz/CYP include fostering new national and international collaborative projects to advance research on *Y. pestis* in Brazil. Strengthening partnerships with reference laboratories and research institutions will facilitate the development of innovative diagnostic methodologies, enhance epidemiological surveillance strategies, and support evolutionary studies of the bacterium (Figure 3).

Addressing these challenges and achieving these goals will not only enhance the scientific value of the Fiocruz/CYP collection

but also reinforce its role as a pivotal resource for public health, biosafety, and global plague research. Furthermore, as one of the most significant *Y. pestis* collections in South America, its continued development contributes to strengthening regional expertise in pathogen surveillance, biodiversity studies, and high-containment microbiology. By fostering integrative research initiatives across the Global South, the collection serves as a critical platform for collaboration, innovation, and capacity-building in the study of zoonotic diseases, ultimately supporting global health preparedness and response efforts.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

None declared.

DATA AVAILABILITY

The data underlying this study are available in a public data repository (http://cyp.fiocruz.br/). Additional details are available from the corresponding author upon reasonable request. The underlying data for this article are available within the manuscript.

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