

CARACTERIZAÇÃO DE BACTÉRIAS ISOLADAS DE AMBIENTES DE PRODUÇÃO FARMACÊUTICA E DEPÓSITO NA COLEÇÃO DE BACTÉRIAS DO AMBIENTE E SAÚDE (FIOCRUZ/CBAS)

CHARACTERIZATION OF BACTERIA FROM PHARMACEUTICAL PRODUCTION ENVIRONMENTS AND DEPOSITED IN THE COLEÇÃO DE BACTÉRIAS DO AMBIENTE E SAÚDE (FIOCRUZ/CBAS)

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RESUMO

Introdução: A presença de microrganismos em ambientes farmacêuticos representa um desafio crítico para o controle microbiológico, podendo impactar a qualidade e a segurança dos produtos.

Objetivo: Caracterizar bactérias isoladas de ambientes farmacêuticos e depositadas na Coleção de Bactérias do Ambiente e Saúde (CBAS) da Fundação Oswaldo Cruz.

Método: Foram depositadas 410 cepas isoladas de diferentes tipos de amostras de duas instalações de produção farmacêutica, provenientes principalmente de soluções intermediárias de processo (n=93), biocarga (n=41), produtos intermediários (n=35), água purificada (n=33) e água potável (n=28), entre outras. Destas, 331 foram identificadas por meio do sequenciamento do gene 16S rRNA.

Resultados: Foram identificados 33 gêneros bacterianos, destacando-se *Staphylococcus* (n=118; 35,6%), *Bacillus* (n=67; 20,2%), *Pseudomonas* (n=41; 12,4%) e *Burkholderia* (n=34; 10,3%). O índice de diversidade de Simpson foi de 0,806. A comparação entre as identificações realizadas pelo depositante e pela CBAS demonstrou consistência metodológica.

Conclusões: A CBAS constitui um recurso relevante para a preservação de linhagens bacterianas provenientes da indústria farmacêutica, apoiando aplicações industriais e futuras pesquisas científicas.

Palavras-Chave: Controle microbiológico. Identificação bacteriana. Coleções microbiológicas. 16S rRNA. Indústria farmacêutica.

ABSTRACT

Introduction: The presence of microorganisms in pharmaceutical environments represents a critical challenge for microbiological control, potentially impacting product quality and safety.

Objective: To characterize bacteria isolated from pharmaceutical environments and deposited in the Culture Collection of Bacteria from the Environment and Health (CBAS) of the Oswaldo Cruz Foundation.

Method: A total of 410 strains isolated from different types of samples from two pharmaceutical production facilities were deposited, mainly from intermediate process solutions (n=93), bioburden (n=41), intermediate products (n=35), purified water (n=33), and potable water (n=28), among others. Of these, 331 were identified by 16S rRNA gene sequencing.

Results: Thirty-three bacterial genera were identified, with *Staphylococcus* (n=118; 35.6%), *Bacillus* (n=67; 20.2%), *Pseudomonas* (n=41; 12.4%), and *Burkholderia* (n=34; 10.3%) being the most prevalent. The Simpson diversity index was 0.806. Comparison between identifications performed by the depositor and CBAS demonstrated methodological consistency.

Conclusions: CBAS constitutes a relevant resource for the preservation of bacterial strains from pharmaceutical industries, supporting industrial applications and future scientific research.

Keywords: Microbiological control. Bacterial identification. Microbial collections. 16S rRNA. Pharmaceutical industry.

1 INTRODUCTION

The pharmaceutical industry operates under strict quality standards to ensure that medicinal products are safe, effective, and free from microbial contamination (Brasil, 2022; EMA, 2022). Therefore, environmental microbiological monitoring is a fundamental component of pharmaceutical quality systems, especially in cleanrooms and controlled manufacturing areas, where the presence of microorganisms may represent a potential risk to product quality and patient safety (Kremer; Patel, 2019; Sutton, 2010; United States Pharmacopeia, 2024). The recovery and characterization of environmental isolates provide valuable information regarding the microbial communities associated with pharmaceutical facilities, contributing to contamination source tracking, process optimization, and the implementation of effective contamination control strategies (EMA, 2022; Sutton, 2012; United States Pharmacopeia, 2025).

Microorganisms frequently recovered from pharmaceutical environments comprise a diverse microbial community, including species belonging to the genera *Staphylococcus*, *Bacillus*, *Micrococcus*, *Acinetobacter* and *Pseudomonas*, among others (Caldeira *et al.*, 2024; Costa *et al.*, 2022; Li *et al.*, 2026; Ramos *et al.*, 2024; Song *et al.*, 2024; Vasconcellos *et al.*, 2023, 2026). Accurate identification of these microorganisms is essential not only to comply with regulatory requirements, but also to understand their ecological distribution, physiological characteristics, and potential impact on pharmaceutical manufacturing processes. Thus, the characterization of microbial isolates has become an important tool for risk assessment and the implementation of preventive measures in quality assurance programs (EMA, 2022; Nunes Ramos *et al.*, 2025; United States Pharmacopeia, 2024).

Traditionally, microbial identification has been based on phenotypic characteristics, including colony morphology, growth patterns, biochemical reactions, and Gram-staining. However, these approaches may present limitations due to phenotypic variability among closely related species (Nunes Ramos *et al.*, 2025). The incorporation of advanced technologies, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and molecular approaches based on DNA sequencing (as targeting 16S rRNA gene), has significantly improved the accuracy, speed, and reliability of bacterial identification (Alizadeh *et al.*, 2020; Church *et al.*, 2020; de Souza *et al.*, 2025).

In this context, the polyphasic approach has become a robust strategy for accurate bacterial identification. The combination of different analytical methodologies minimizes the limitations associated with individual techniques and provides greater confidence in taxonomic

assignment, especially when studying environmental microorganisms with considerable phenotypic diversity (Nunes Ramos *et al.*, 2025). In pharmaceutical industries, integrating proteomic (MALDI-TOF MS) and whole genome sequencing (WGS) have been used to achieve a fast and accurate identification and also been useful for MALDI-TOF MS expansion database and microbial contamination tracking (Andrade *et al.*, 2025; De Miranda *et al.*, 2023; Li *et al.*, 2026; Song *et al.*, 2024; United States Pharmacopeia, 2025).

Although environmental monitoring programs in pharmaceutical industries routinely generate a large number of microbial isolates, these microorganisms are often typically targeted for immediate identification for contamination investigations with limited efforts directed toward their long-term preservation and systematic organization (Kremer; Patel; 2019; Ramos *et al.*, 2024). Nevertheless, environmental isolates represent valuable biological resources, providing information about microbial diversity, temporal changes in environmental microbiota, and some of them can also possess potential for biotechnological application (Li *et al.*, 2026; Song *et al.*, 2024),

The establishment and maintenance of microbial collections play a fundamental role in preserving microbial biodiversity and ensuring the availability of well-characterized strains for future scientific investigations. Biological collections function as repositories of genetic and phenotypic information, supporting studies in taxonomy, evolution, ecology, biotechnology, and the development and validation of analytical methodologies (Arez *et al.*, 2024; da Silva; Chame; Moratelli, 2020; OECD, 2007; WFCC, 2010). In the pharmaceutical context, the preservation of environmental isolates may also contribute to investigations, improvement of microbial identification databases, using as in house strains for validation of microbiological methods and cleaning and disinfection procedures (Andrade *et al.*, 2025; Costa *et al.*, 2026; Rhodes *et al.*, 2025, 2026; Valadão *et al.*, 2026).

Considering the importance of understanding microbial diversity in pharmaceutical environments and the need to preserve representative strains for future applications, the present study aimed to describe the profile of bacteria strains isolated from pharmaceutical industry facilities and deposited in the Bacteria Collection of Environment and Health (CBAS) from Oswaldo Cruz Foundation (Fiocruz).

2 METHODS

2.1 BACTERIAL STRAINS

Four hundred and ten (n=410) bacterial isolates were recovered from different types of samples of two manufacturing pharmaceutical facilities. Following preliminary identification performed by the depositors, the strains were deposited to the CBAS for preservation and subsequent taxonomic characterization. CBAS is hosted at Fiocruz, Rio de Janeiro, Brazil (www.cbas.fiocruz.br), affiliated with the World Federation for Culture Collections (WFCC) and registered as World Data Centre for Microorganisms (WDCM) 958. After data screening and organization, the selected strains were incorporated into the pharmaceutical subcollection of the CBAS.

2.2 PHENOTYPIC CHARACTERIZATION

After deposition in the CBAS, the bacterial isolates were subjected to preliminary phenotypic characterization. Gram-staining, colony morphology assessment, and photographic documentation after 24 and 48 h of growth were performed on cultures cultivated in media recommended by the depositor. In the present study, the culture media used were TSA (Tryptic Soy Agar) (KASVI, São José dos Pinhais, Brazil) and BA (Blood Agar) (Biocen, São Paulo, Brazil).

The macroscopic characteristics evaluated included colony shape, pigmentation, texture, margin, and elevation, while Gram-staining provided the initial differentiation between Gram-positive and Gram-negative bacteria.

2.3 16S rRNA GENE AMPLIFICATION, SEQUENCING AND TAXONOMIC IDENTIFICATION

Bacterial identification was performed by amplification and sequencing of the nearly complete 16S rRNA gene (~1,500 bp). Genomic DNA was obtained from pure bacterial cultures either by thermal lysis or using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. For thermal lysis, bacterial biomass was suspended in 500 µL of sterile DNase- and RNase-free water, homogenized for approximately 1 min, subjected to boiling for 15 min, and immediately stored at $\leq -15^{\circ}\text{C}$ for at least 4 h. The suspension was subsequently thawed and centrifuged at $13,000 \times g$ for 1 min, and the supernatant was used as the DNA template for PCR.

PCR amplification was carried out in a final reaction volume of 50 µL, consisting of 10 µL of 5× PCR buffer, 6 µL of MgCl_2 (25 mM; final concentration of 3 mM), 0.3 µL of Taq

DNA polymerase (5 U/ μ L; final amount of 1.5 U per reaction), 1 μ L of dNTPs (10 mM; final concentration of 0.2 mM), 1 μ L of forward primer (pAF or pH; 100 ng/ μ L), 1 μ L of reverse primer (1492R, 1525R or 1093R; 100 ng/ μ L), and DNase- and RNase-free water to complete 47 μ L of reaction mixture. Subsequently, 3 μ L of DNA template was added, resulting in a final volume of 50 μ L. PCR conditions consisted of an initial denaturation step at 95 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min and 30 s, followed by a final extension at 72 °C for 10 min.

PCR products were analyzed by agarose gel electrophoresis using 5–7 μ L of amplified product mixed with 2 μ L of sample loading buffer and 2 μ L of GelRed® (UNISCIENCE, São Paulo, Brazil) in 1 $\times$ TAE buffer. Electrophoresis was performed at approximately 60 V for at least 1 h, and DNA bands were visualized under ultraviolet light using a gel documentation system. The expected amplicon size was approximately 1,500 bp, estimated by comparison with a molecular weight marker. PCR products were purified using the Wizard®SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). Purified amplicons were subjected to Sanger sequencing using BigDye Terminator chemistry and analyzed on a 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at the DNA Sequencing Platform of the Oswaldo Cruz Foundation (Fiocruz, Rio de Janeiro, Brazil).

The obtained sequences were edited and assembled using SeqMan® software (DNASTAR Lasergene, version 7.0.0) and subsequently compared against the EzBioCloud database (Chalita *et al.*, 2024) for taxonomic identification. Genus-level assignment was performed based on 16S rRNA gene sequence similarity values of $\geq 95\%$.

2.4 BACTERIAL DIVERSITY ANALYSIS

The bacterial diversity of the collection was evaluated using the Simpson diversity index (1–D), as described by Grundmann *et al.* (2001). This index estimates the probability that two isolates randomly selected from the collection belong to different bacterial genera. Values range from 0 to 1, with values closer to 1 indicating greater bacterial diversity.

The Simpson diversity index was calculated using the following equation:

$$1 - D = 1 - \frac{\sum_{i=1}^S n_i(n_i - 1)}{N(N - 1)}$$

where S is the total number of identified bacterial genera, n_i is the number of isolates belonging to the i -th genus, and N is the total number of identified isolates.

The relative frequency of each bacterial genus (p_i) was calculated as:

$$p_i = \frac{n_i}{N}$$

The variance of the diversity index (σ^2) was estimated according to Grundmann et al. (2001):

$$\sigma^2 = \frac{1}{N} \left[\sum_{i=1}^S p_i^3 - \left(\sum_{i=1}^S p_i^2 \right)^2 \right]$$

where p_i is the relative frequency of the i -th bacterial genus.

The 95% confidence interval (95% CI) was estimated as:

$$CI = [(1 - D) - 2\sqrt{\sigma^2}, (1 - D) + 2\sqrt{\sigma^2}]$$

where CI represents the confidence interval of the Simpson diversity index and σ^2 is the estimated variance.

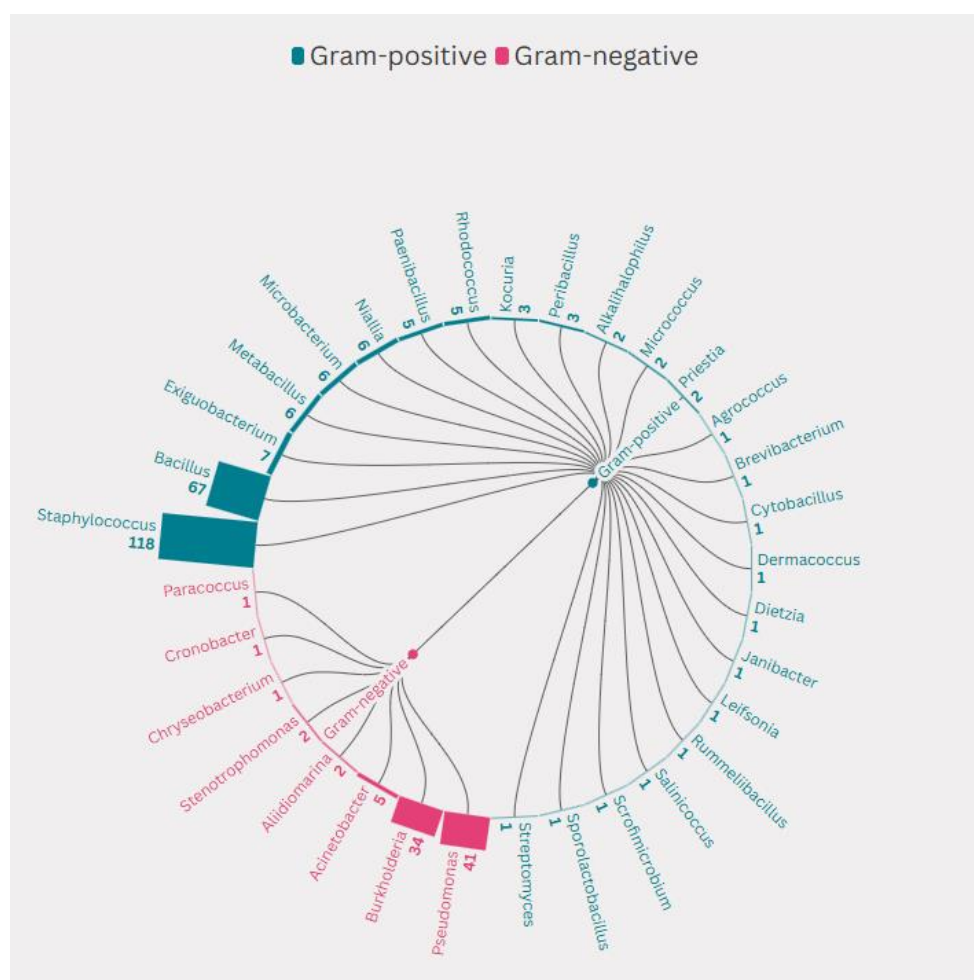
3. RESULTS AND DISCUSSION

A total of 410 bacterial strains were deposited in the CBAS, representing strains recovered from different pharmaceutical environments and matrices. Among these, 331 (80.73 %) strains were identified into 33 different genera (Figure 1), a proportion of ~10 strains/genus, by 16S rRNA sequencing. The calculated SI was 0.806 (0.776–0.835), demonstrated the taxonomic diversity of the strain from the pharmaceutical environments deposited in the CBAS.

Among the 410 bacterial strains deposited in the CBAS, 331 isolates were identified, while 79 strains remained unidentified at the time of manuscript preparation. This was primarily due to the ongoing characterization process of recently deposited isolates, including strains recovered between 2021 and 2026. Some isolates also produced low-quality or inconclusive sequencing results, requiring additional analyses before taxonomic assignment. As the CBAS pharmaceutical subcollection continues to receive and characterize new bacterial strains, additional identification results are expected to be incorporated in future updates of the collection.

Although 16S rRNA gene sequencing was effective for the preliminary characterization and taxonomic assignment of most bacterial isolates deposited in the CBAS, further improvements in the identification workflow may enhance the taxonomic resolution of the collection. Since closely related bacterial species may exhibit highly similar 16S rRNA gene sequences, complementary approaches, such as whole-genome sequencing (WGS), genomic analyses, and expanded proteomic databases, should be considered as future perspectives for the comprehensive characterization of pharmaceutical-associated isolates preserved in the CBAS.

Figure 1 – Distribution of bacterial genera and Gram characteristics among pharmaceutical isolates deposited in the CBAS



Legend: Sunburst chart illustrating the distribution of the 331 identified bacterial isolates according to bacterial genus and Gram staining characteristics. The inner ring represents the Gram staining classification, whereas the outer ring shows the distribution of bacterial genera.

Source: Prepared by the authors.

The most frequently identified genera were *Staphylococcus* (n = 118; 35.6%), *Bacillus* (n = 67; 20.2%), *Pseudomonas* (n = 41; 12.4%), and *Burkholderia* (n = 34; 10.3%), which

together represented the majority (78,5%) of the identified isolates (Figure 1). In addition, the presence of several genera with lower frequencies demonstrates the broad taxonomic diversity preserved within the collection, including microorganisms associated with different pharmaceutical environments and matrices.

Regarding the phenotypic characterization, a predominance of Gram-positive bacteria was observed, corresponding to 244 (73.7%) strains, while Gram-negative bacteria represented 87 (26.3%). The predominance of Gram-positive bacteria may be associated with their ability to survive under the environmental conditions commonly found in pharmaceutical facilities, including low nutrient availability, desiccation stress, and frequent exposure to cleaning and disinfection procedures, particularly members of the genera *Staphylococcus* and *Bacillus* (Cogo-Müller, 2024; Costa *et al.*, 2022; Li *et al.*, 2026; Sheraba; Yassin; Amin, 2011; Soares; Amorim-Neto; Song *et al.*, 2024). These characteristics highlight the importance of preserving and characterizing such isolates to support contamination assessment, microbiological monitoring, and future pharmaceutical quality control strategies. Although the classification of the pharmaceutical areas where the isolates were recovered could provide additional insights into the relationship between microbial distribution and environmental criticality, this analysis was not performed due to institutional confidentiality restrictions regarding manufacturing areas and processes. The higher occurrence of *Staphylococcus*, *Bacillus*, *Pseudomonas*, and *Burkholderia* suggests that pharmaceutical environments harbor a characteristic microbiota composed of microorganisms repeatedly recovered during environmental monitoring programs. Similar distributions have been reported by Song *et al.* (2024) and Li *et al.* (2026), who also described the predominance of genera such as *Staphylococcus*, *Bacillus*, *Pseudomonas*, and *Burkholderia* in pharmaceutical manufacturing environments. In the present study, *Staphylococcus* and *Bacillus* were among the most frequently identified genera, which may be related to their adaptation to dry environments, frequent human contact, and resistance to environmental stress conditions. *Staphylococcus* is commonly associated with personnel monitoring, air samples, and surface contamination, whereas *Bacillus* species are frequently recovered from air, surfaces, and raw materials due to their ability to produce highly resistant endospores (Mattoso *et al.*, 2025). In contrast, Gram-negative genera such as *Pseudomonas* and *Burkholderia* were mainly associated with water-related samples, reflecting their capacity to survive in low-nutrient aquatic systems and to form biofilms (Silva-Santana *et al.*, 2025; Vasconcellos *et al.*, 2026). Although these dominant genera represented the majority of isolates, the presence of several less frequent genera demonstrates that pharmaceutical environments

maintain a broader microbial diversity that should also be considered in contamination control strategies.

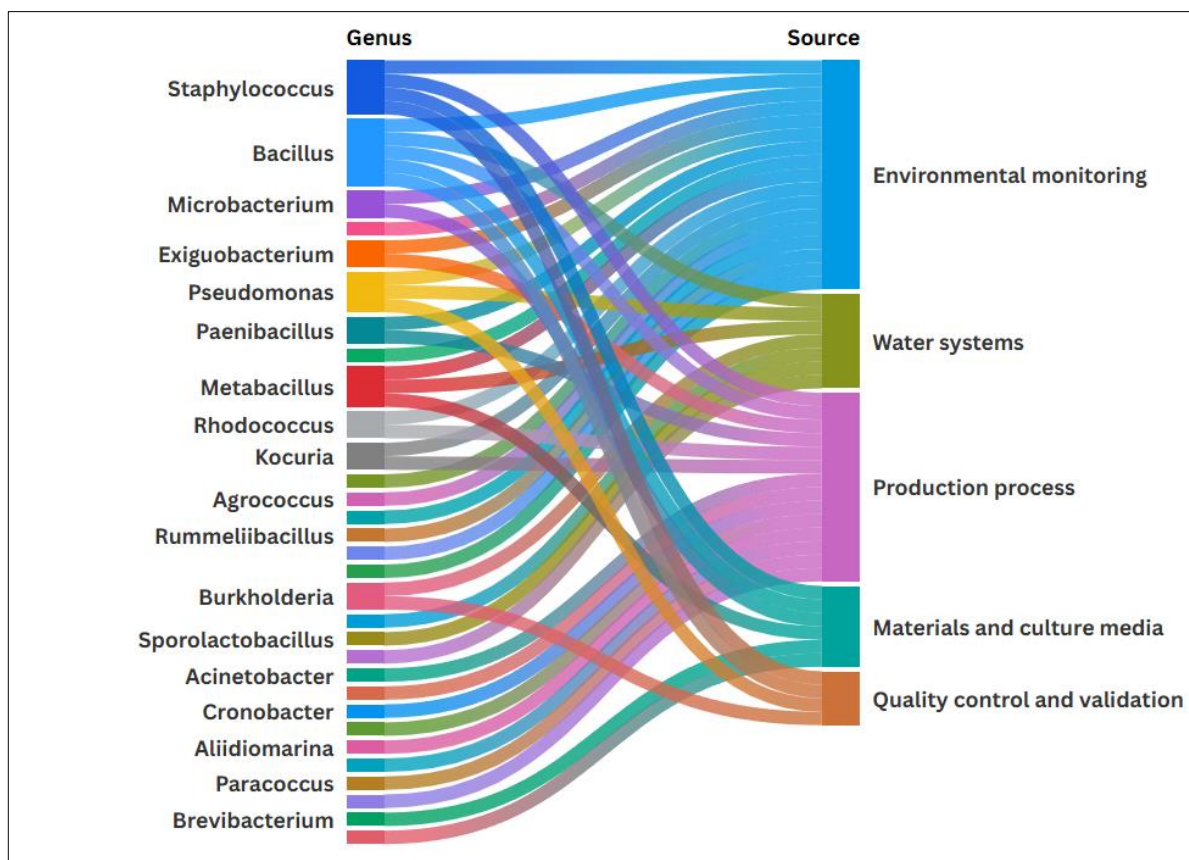
The coexistence of dominant and low-frequency genera reinforces the value of CBAS as a repository of both recurrent and less commonly recovered microorganisms. Preserving this diversity is important not only for taxonomic studies, but also for future investigations related to environmental monitoring, contamination control, and microbial risk assessment in pharmaceutical settings. In addition, studies performed by pharmaceutical industries using in house strains that were deposited in CBAS were already reported, demonstrating the importance of maintaining of these strains and the authentication realized by CBAS (Andrade *et al.*, 2025; Costa *et al.*, 2026; Rhodes *et al.*, 2025, 2026; Valadão *et al.*, 2026). The preservation of environmental isolates in the CBAS expands their potential applications beyond long-term conservation. For example, an environmental isolate deposited in the collection has already been used in a validation study to evaluate the inhibitory activity of collection vials for high-temperature pharmaceutical water analysis (Costa *et al.*, 2023). Furthermore, preserved environmental isolates may support microbiological method validation, growth promotion testing, contamination source tracking, cleaning validation, and investigations of environmental monitoring deviations, since they better represent the microbiota encountered during routine pharmaceutical manufacturing than standard reference strains. In addition, the deposition of authenticated strains in culture collections is essential for the description of new species, as demonstrated for *Bacillus lumedeiriae* (Costa *et al.*, 2024), *Brachybacterium manguinensis* (Caldeira *et al.*, 2026), and *Lederbergia fraccalanzzae* (Ramos *et al.*, 2026), all isolated from pharmaceutical facilities. Beyond their taxonomic relevance, strains preserved in collections can also support applied microbiological studies. For example, the Main Spectrum Profile (MSP) of the strain CBAS1225^T, *Bacillus lumedeiriae* sp. nov. type strain, was incorporated into a local library of the MALDI Biotyper[®] database, contributing to the expansion of microbial identification databases and improving the identification capacity of microorganisms in a pharmaceutical microbiological quality control laboratory (Costa *et al.*, 2024). These applications highlight the importance of maintaining authenticated and well-characterized microbial collections, such as CBAS, as strategic resources for contamination investigations, microbial identification, and quality control activities in pharmaceutical environments.

The bacterial isolates preserved in the CBAS originated from two pharmaceutical manufacturing facilities and represent samples collected between 2021 and 2026. Although a direct comparison between the facilities was not performed, the dataset encompasses microorganisms recovered from diverse pharmaceutical contexts, including environmental

monitoring, water systems, production-related samples, and quality control activities. This broad representation allows the assessment of bacterial diversity associated with different pharmaceutical operational environments while maintaining institutional confidentiality regarding specific facility characteristics.

A Sankey diagram illustrates the distribution of the 331 identified bacterial genera across the different types of samples.

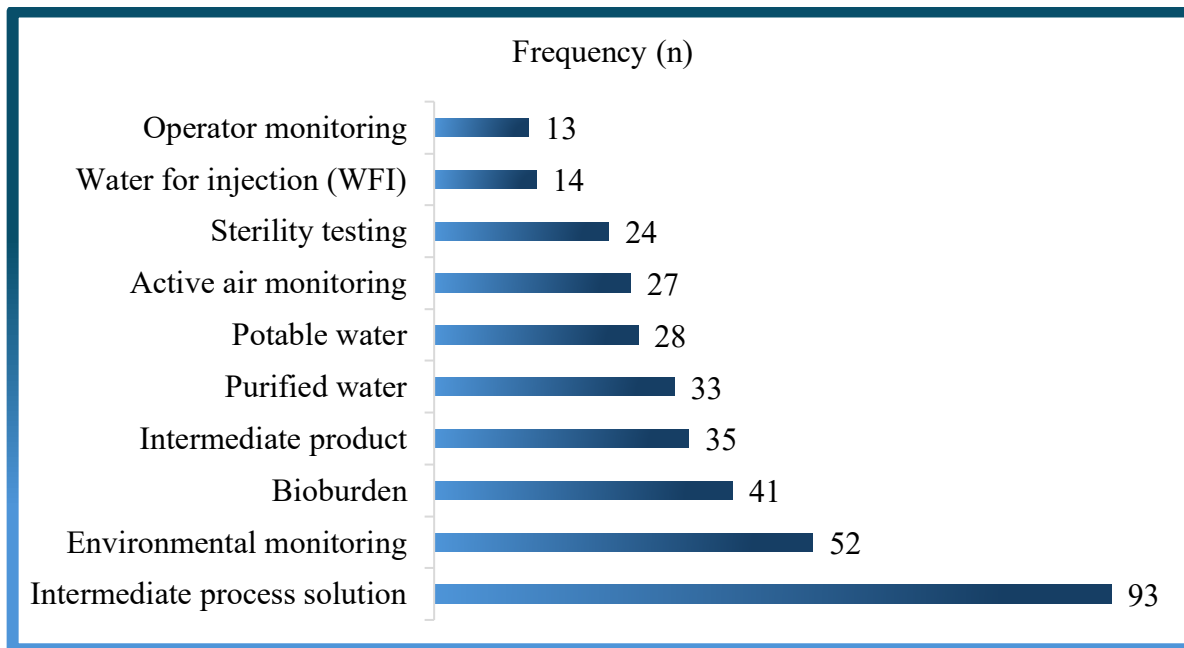
Figure 2 – Sankey diagram illustrating the relationship between the isolation source in the pharmaceutical industry and bacterial genera identified in the CBAS



Legend: The width of the streams represents the proportion of each workflow, with isolation sites grouped into broader categories for visualization purposes. The sampling sources were grouped as follows: Environmental monitoring – environmental monitoring, active air monitoring, operator monitoring, and contact monitoring; Water systems – purified water, potable water, water for injection (WFI), softened water, water, and water monitoring; Production process – intermediate product, intermediate process solution, and finished product; Materials and culture media – cell culture, culture medium, and SFM4CHO culture medium; Quality control and validation – bioburden, sterility testing, investigation, utility validation, process validation, media fill, aseptic process, aseptic simulation, active pharmaceutical ingredient (API), raw material, filter swab, and inoculation (API).

Source: Prepared by the authors.

Figure 3 – Distribution of bacterial isolates among the ten most frequent sampling sites represented in the CBAS collection



Source: Prepared by the authors.

The distribution of isolates according to sampling source revealed that the CBAS collection encompasses microorganisms recovered from multiple stages of pharmaceutical manufacturing and quality control activities. The highest frequencies were observed for intermediate process solutions (n=93, 22.68%), environmental monitoring samples (n=52, 12.68%), bioburden analyses (n=41, 10%), and intermediate products (n=35, 8.54%), demonstrating that the collection is not restricted to routine environmental monitoring but also includes microorganisms associated with production processes. This broad representation is further illustrated by the Sankey diagram (Figure 2), which shows the occurrence of the predominant genera across environmental monitoring, water systems, production-related samples, and quality control activities.

An important strength of the present study is the broad representation of bacterial strains from pharmaceutical environments preserved in the CBAS. Previous studies have predominantly focused on microorganisms recovered from specific pharmaceutical environments, such as environmental monitoring, water systems, or particular contamination events (Li *et al.*, 2026; Mattoso *et al.*, 2025; Song *et al.*, 2024). In contrast, the present study brings together isolates recovered from multiple pharmaceutical sources, including environmental monitoring, water systems, production processes, intermediate products, and quality control activities, providing a more comprehensive representation of the microbial

diversity associated with pharmaceutical manufacturing environments. This diversity expands the scientific value of the collection and provides a reliable source of well-characterized strains for future taxonomic, ecological, and applied microbiology studies. Furthermore, preserving these authenticated isolates in the CBAS supports improvements in microbial identification, contamination control strategies, environmental monitoring programs, and the development of reference databases for the pharmaceutical industry.

Environmental monitoring programs are traditionally considered one of the main sources of microbial recovery in pharmaceutical facilities (Sutton, 2010, 2012; USP, 2024). However, the results obtained in this study highlight the relevance of process-related samples and water systems as important reservoirs of microbial diversity. Similar observations have been reported in studies involving microbial characterization in pharmaceutical environments, where genera such as *Staphylococcus*, *Bacillus*, *Pseudomonas*, and *Burkholderia* were recovered from environmental monitoring samples, water systems, and manufacturing-related materials (Cogo-Müller, 2024; Li *et al.*, 2026; Mattoso *et al.*, 2025; Soares; Amorim-Neto;; Song *et al.*, 2024). The presence of these microorganisms in distinct sampling sources reinforces the importance of comprehensive monitoring strategies and supports the value of maintaining well-characterized microbial repositories representative of the different niches found within pharmaceutical facilities.

Among the studies analyzed, environmental monitoring and water-related samples were the most frequently reported sources of bacterial isolation in pharmaceutical facilities. Environmental monitoring programs, including air, surface, and clean-room monitoring, were described in several studies (Caldeira *et al.*, 2024; Sheraba *et al.*, 2010; Soares *et al.*, 2024; Song *et al.*, 2024), while water systems were also highlighted as important reservoirs of pharmaceutical microorganisms (Caldeira *et al.*, 2024; Silva-Santana *et al.*, 2025; Vasconcellos *et al.*, 2026). In the present study, however, the highest frequency of isolates was observed in intermediate process solutions (22.68%), followed by environmental monitoring samples (12.68%) and bioburden analyses (10.00%). This profile suggests that the CBAS collection is not limited to microorganisms recovered through routine environmental monitoring, but also preserves a substantial number of strains associated with production processes and quality-control activities, thereby broadening the diversity of ecological niches represented in the collection.

The CBAS is a bacteria collection from IOC/Fiocruz and is affiliated with the WFCC under registration code WDCM 958. The collection is managed by a designated curatorial structure, with Verônica Viana Vieira as curator and Cátia Aparecida Chaia de Miranda as

deputy curator. CBAS follows principles related to biological resource management, including documentation, characterization, preservation, and quality control of deposited bacterial strains. The bacterial isolates are characterized through phenotypic and genotypic approaches, including photographic records of colony morphology, Gram staining, and 16S rRNA gene sequencing for taxonomic identification. Cryopreservation is performed using different preservation media, including Brain Heart Infusion Broth (BHI) supplemented with 20% glycerol, Tryptic Soy Broth (TSB) supplemented with 15% glycerol, and skim milk. Preserved strains are maintained at ultra-low temperatures (-80°C) and in liquid nitrogen, with multiple cryovials established for each strain, including master, liquid nitrogen, memory, reserve, and working stocks. Viability and purity tests are performed during the preparation of cryopreserved stocks to ensure the quality of preserved resources. Access to CBAS strains is managed through institutional procedures, with information available through the collection catalog and requests submitted through official forms. Strain distribution is evaluated according to the intended scientific purpose and the qualification of the applicant, ensuring appropriate use of authenticated biological resources. These practices support the long-term preservation, traceability, and scientific use of bacterial strains maintained by the CBAS.

4 CONCLUSIONS

This study characterized bacterial strains isolated from pharmaceutical production environments and preserved in the CBAS, demonstrating the taxonomic diversity associated with pharmaceutical manufacturing facilities. The predominance of the genera *Staphylococcus*, *Bacillus*, *Pseudomonas*, and *Burkholderia*, together with the diversity of sampling sources represented in the collection, highlights the representativeness of the preserved microorganisms.

The establishment of this pharmaceutical subcollection expands the availability of well-characterized environmental isolates for future scientific and technological applications, including microbial identification, contamination investigations, environmental monitoring, microbiological quality control, and the validation of analytical methods. Therefore, the CBAS constitutes a valuable biological repository that supports microbiological research and contributes to improving contamination control strategies in pharmaceutical industries.

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