

THE CULTURE COLLECTION OF PHYTOPATHOGENIC MICROORGANISMS: AN IMPORTANT SOURCE OF INFORMATION TO COMMON BEAN RESEARCH

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The culture collection of common bean pathogenic microorganisms of Embrapa Rice and Beans was created in the 1980's aiming to estimate the variability of the major pathogens and thus guide the achievement of resistant cultivars. Currently the collection has more than 4.000 isolates of bean pathogens, being the most relevant species: *Colletotrichum lindemuthianum*, *Pseudocercospora griseola*, *Fusarium oxysporum f. sp. phaseoli*, *Sclerotinia sclerotiorum*, *Uromyces appendiculatus*, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* and *Xanthomonas axonopodis* pv. *phaseoli*. With the creation of the Embrapa Microbial Genetic Resources Network in 2010, evidenced the importance of the collection in the context of the Brazilian genetic heritage and with the financial support made possible the cataloging, maintenance and monitoring more appropriate for the collection. The challenge now is the implementation of the electronic database and a search system, available to internal and external audiences, with the most relevant information to users, including data obtained with the use of molecular markers. Thus, in addition to facilitating access to information contained in the collection to the public internal company also will facilitate access to information by other external research groups, contributing more strongly to the development of research related to pathosystems. The objective of this study was to group the various data relating to the common bean pathogenic microorganisms from Embrapa Rice and Beans culture collection, aiming to emphasize its complexity and importance to the development of disease-resistant plants. Samples from several common beans producing regions of Brazil were received over 30 years (1981-2011) by the Laboratory of Plant Pathology at Embrapa Rice and Beans. The data collection of each material was carefully recorded in the minute book containing collector name, collection site, collection date, and georeferenced cultivar information. The plant material was evaluated on the types of symptoms, disinfection with 70% ethanol and diluted sodium hypochlorite was done. The isolation of pathogens was performed differently for bacteria and fungi. For the isolation of bacteria the sample was macerated and the formed suspension was inoculated in Petri dishes containing culture medium potato dextrose agar (PDA) or nutrient agar, depending on the type of disease. For the isolation of fungi it was used the technique for microorganism monosporic and inoculation in Petri dish with BDA medium. Both bacteria and fungi strains were incubated at specific temperatures for each pathogen. After morphological, biochemical and molecular characterization, the identified isolates were subjected to long-term preservation in three different methods: cryopreservation, Castellani (in water) and filter paper. Besides, isolates were also characterized according to their pathogenicity by using a differential cultivar series according to each pathogen (CIAT, 1990; Rava & Sartorato, 1994, Pastor-Corrales & Abawi, 1987) or by specific molecular detection (Tegli et al. 2002, Alves-Santos et al., 2002).

The bean pathogens collection grew uninterrupted since 1981 due to the constant sampling of plant material with symptoms of disease, obtained from many common bean production regions in Brazil. Along the thirty years it has been obtained over 3500 isolates from seven different species of pathogens that cause diseases in common bean plants (Figure 1). All pathogenic microorganisms were evaluated morphologically to observe the spores shape and size (i.e. conidia of *C. lindemuthianum*) and the cell wall structure (i.e. gram positive bacteria of the specie *C. flaccumfaciens* pv. *flaccumfaciens*) (Figure 2 A and B). Some of them were also evaluated using molecular marker, resulting on the clustering of the isolates (Figure 2C). Molecular and

pathogenicity data indicated a high degree of variability among the isolates of the same species and the predominance of certain pathotypes according to their origin regions, showing the need for sampling and continuous isolation over the years. The isolates are used mainly for the selection of disease resistant genotypes, pathogen-host interaction studies and on the characterization of physiological races. The on line query and request of common bean pathogenic isolates can be found at the National Research Center of Rice and Beans website http://www.cnpaf.embrapa.br/transferecia/informacoestecnicas/colecao_fitopatogenos/index.php

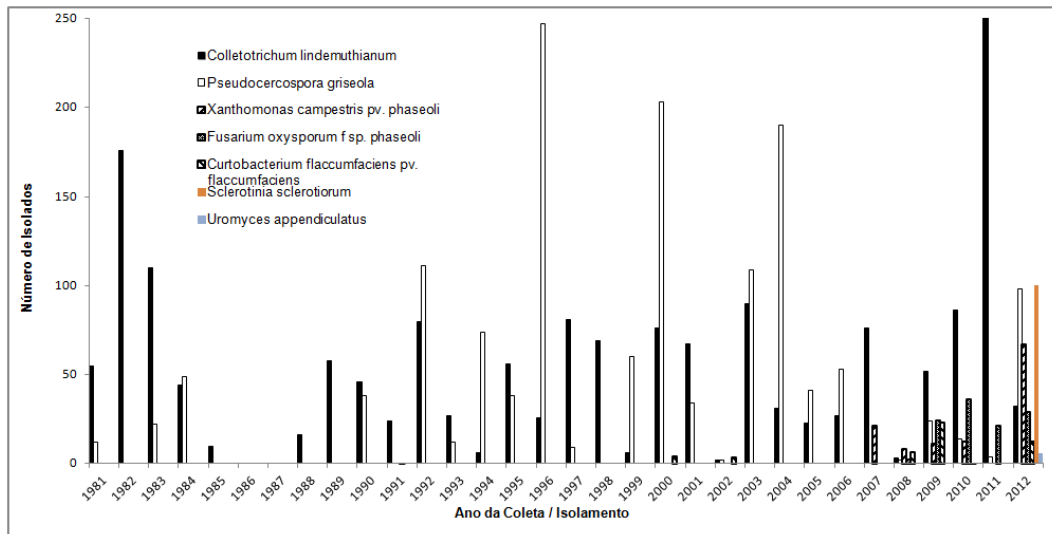


Figure 1. Number of different pathogenic species obtained between the years 1981 and 2012.

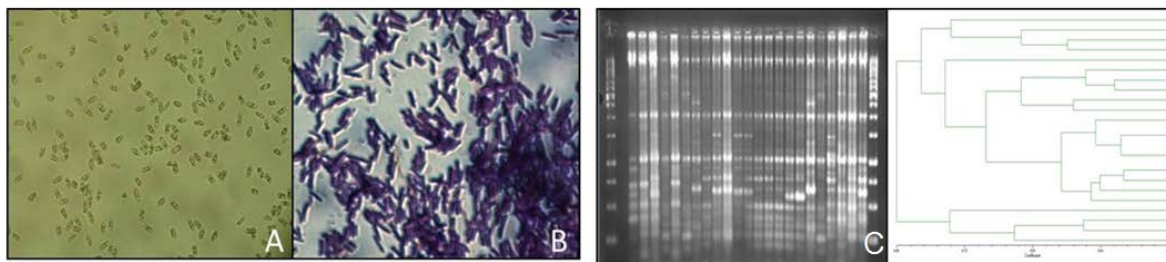


Figure 2. Morphological characterization: A) conidia of *C. lindemuthianum*, B) gram positive bacteria of the species *C. flaccumfaciens* pv. *flaccumfaciens*. C) Molecular characterization of different isolates of *C. flaccumfaciens* pv. *flaccumfaciens*.

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