Note

Sexual reproduction in subcultures of *Absidia blakesleeana* after years of preservation under mineral oil

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Summary Two subcultures of *Absidia blakesleeana* IOC No. 2425 (–) mating type strain preserved in 1959 and 1981 on a thick layer of potato dextrose agar (PDA) medium under sterile mineral oil and maintained at room temperature in the Fungal Cultural Collection of Institute Oswaldo Cruz –IOC– were paired with the defined *Absidia blakesleeana* URM-UFP No. 2076 (+) mating type strain. Portions of the line where mycelia of the two strains met were observed under light microscopy. Zygospores belonging to Subgenus *Mycocladus* were observed. The results demonstrated conservation and re-establishment of the physiological and genetic metabolic processes of two subcultures preserved under mineral oil.

Key words *Absidia blakesleeana*, Sexual reproduction, Preservation, Mineral oil

Reproducción sexual en subcultivos de *Absidia blakesleeana* preservados en aceite mineral

Resumen Se acoplaron dos subcultivos de *Absidia blakesleeana* IOC N.º 2425 de la cepa del tipo acoplador (–) preservados en 1959 y en 1981 en un medio de espesa camada de patata dextrosa agar (PDA) bajo aceite mineral estéril y mantenidos a temperatura ambiente en la Colección de Hongos del Instituto Oswaldo Cruz-IOC, con la cepa definida de *Absidia blakesleeana* URM-UFP N.º 2076 de tipo acoplador (+). Después del acoplamiento, se observaron en el microscopio los segmentos de la línea donde se unieron micelios de ambos tipos. Se observaron cigosporas del subgénero *Mycocladus*. Los resultados demostraron la conservación y el restablecimiento de los procesos genéticos y fisiológicos dos subcultivos de tipos de *Absidia blakesleeana* preservadas en una espesa camada de un medio de patata dextrosa agar bajo aceite mineral estéril.

Palabras clave *Absidia blakesleeana*, Reproducción sexual, Preservación, Aceite mineral

Sexual reproduction in heterothallic species of the order Mucorales proceeds when two compatible individualistic mycelia [11] of a (+) and (–) mating type strains are paired under suitable conditions giving rise to mature and/or immature zygospores. The genus *Absidia* contains species whose zygospores are divided into two subgenera: *Absidia* subgenus *Mycocladus* for species whose zygospores are not surrounded/enveloped by appendages from the suspensors, and *Absidia* subgenus *Absidia* for species whose zygospores are surrounded/enveloped by appendages from the suspensors [12].

The scientific process of conserving and maintaining the biological heritage of a culture collection of filamentous fungi under mineral oil method dates back to 1943 [13]. Advantages of its application in a public or private culture collection of fungi are: easy transfer of the cultures conserved in this way to fresh media, low cost and exceedingly simple procedure [3].

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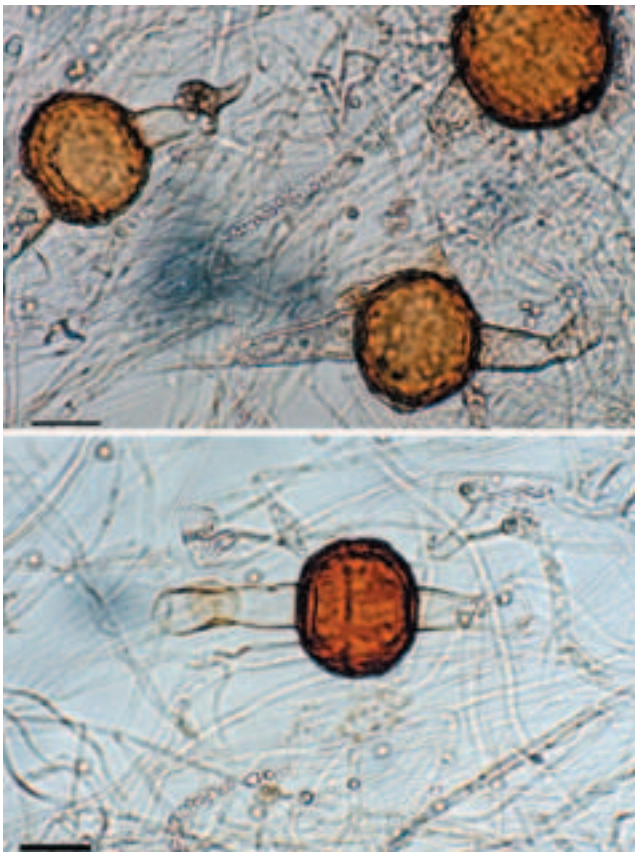

Figure 1. Zygospores of *Absidia blakesleeana* IOC. strain No. 2425 on YM agar; bar: 25 µm.

This study describes the results of intraspecific crosses of two subcultures of *Absidia blakesleeana* IOC-FIOCRUZ No. 2425 (–) mating behavior strain which were preserved in 1959 and 1981 on a thick layer of potato dextrose agar (PDA) medium under sterile mineral oil and maintained at room temperature with the defined *Absidia blakesleena* URM-UFP 2076 (+) mating type strain.

One stock culture of *Absidia blakesleeana* IOC No. 2425 (–) mating type strain, received by the Fungal Culture Collection of Istituto Oswaldo Cruz – IOC in 1947 was subcultured in 1959 and 1981 and preserved on a thick layer of potato dextrose agar (PDA) medium under sterile mineral oil and maintained at room temperature in the collection. After macro and microscopic examination [16], these two axenic living subcultures were paired about 5 cm apart with the defined *A. blakesleeana* URM-UFP No. 2076, (+) mating type strain, on Petri dishes containing 10-15 ml of MY agar [5], incubated at 31 °C under alternating cycles of 12 h light and 12 h darkness. The Petri dishes were examined under a stereoscopic binocular microscope on each day throughout a period of 7 to 15 days after crossing to inspect for zygospore formation. Preparations mounted in Amann lactophenol solution were prepared on the portions of the line where mycelia of the two strains met, and were observed by light microscopy. Zygospores Subgenus *Mycocladus* [12] developed after 7 days along the line where the two mycelia met. They are 42-76 µm diameter; sometimes compressed between suspensors; yellow brown to dark brown; characterized by an equatorial/broad ridge which circles them at the region of the fusion of the gametangia; suspensors straight and/or bent, arising directly from the aerial mycelium, equal, hyaline to slightly yellowish, roughened, without appendages (Figure 1). Examined under a light microscope, the aspects of these basic units of reproduction were as by Hesseltine & Ellis [6].

According to A.F. Blakeslee, zygospore production in members of the order Mucorales, is conditioned by the inherent nature of the individual species and/or by the individual identity of the mycelium and only secondarily, or not at all, by external factors [1]. The results of this study demonstrated that years of preservation at room temperature on a potato dextrose agar (PDA) medium under a layer of sterile mineral oil, preserved both, the physiological/hormonal and genetic/meiotic system since the heterothallism condition i.e., the (+ and –) mating type behavior, sexual ability and mature zygospores were preserved and developed. This presumably occurred in addition to the physical and metabolic effects on the subcultures during storage, i.e., reduction of available oxygen, decline in the rate of respiration, decreased but continuous mycelial growth and the reduction of staling/toxic products ("oxidative stress") [3,4]. Documented and figured feature studies of zygosporogenesis by light, transmission and scanning electron microscopy are given by O´Donnell et al. [9,10]. Studies concerning sterile mineral oil applicability/preservation for Coelomycetes [2], dermatophytes [14,15] and dimorphic fungi [7,8] have also been published.

The significance of these results are to demonstrate the conservation and re-establishment of the physiological and genetic metabolic processes of subcultures of *Absidia blakesleeana* strain preserved in different periods of time and maintained at room temperature on a thick layer of potato dextrose agar medium under sterile mineral oil.

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