Exophiala psychrophila: A new agent of chromoblastomycosis

Lina M. Garzona,⁎, Lili J. Rueda, Adriana Marcela Celis, Martha Cardenas, Marcela Guevara-Suarez

⁎ Corresponding author.
E-mail address: lgarzon@unbosque.edu.co (L.M. Garzon).

A 25-year-old woman living in a rural area who works as a farmer near a river, without other important antecedents, began to have skin lesions (day 0) on her right ankle; She went to the hospital for the first time (+24 months) where they diagnosed chromoblastomycosis and offered treatment with itraconazole 100 mg/day for three months and subsequent cryotherapy, achieving complete remission (+27 months). One year after the therapy a relapse was observed (+39 months). The patient consulted our hospital 18 years later (+20 years). The physical examination revealed a hypertrophic and warty plaque with healing areas on the right ankle (Fig. 1). Skin biopsy was obtained for histopathological examination, direct microscopic and microbiological analysis. For cytological examination, the skin biopsy was stained with haematoxylin-eosin and this analysis showed chronic granulomatous inflammation with suppurative granulomas (Fig. 2); while direct microscopic examination revealed muriform cells. In addition, we obtained on Saboraud dextrose agar (SDA; Oxoid, UK) colonies of a black fungal. Cultures for bacteria were negative. A second skin biopsy culture was necessary in order to confirm the results found in the previous one. The fungus colonies were selected from the SDA cultures and submitted to the Laboratorio de Micolología y Fitopatología (LAMFU) of the Universidad de los Andes (Bogotá- Colombia) for phenotypic and molecular identification. The isolate was subcultured onto SDA, Mycosel agar (MI), oatmeal agar (OA, BBL, Becton Dickinson and Company, USA), potato dextrose agar (PDA; Oxoid, UK), malt extract agar (MEA; Difco Laboratories, Detroit, MI), oatmeal agar (OA, BBL, Becton Dickinson and Company, USA), and Czapek's agar (CZ; BBL, Becton Dickinson and Company, USA). Phenotypic characterization was carried out using standard growth conditions at 25 °C in the dark. For microscopic observations, slides were made with lactophenol cotton blue using 7–10 days old cultures. We observed, colonies attaining 10–18 mm on MEA, flat, velvety, greenish grey (26F2) and reverse near black (25F4). On microscopic observation dematiaceous septate hyphae, annelids, ellipsoidal conidia emerging at the tip of an annelid were observed. Based upon the microscopic and macroscopic features noted, the isolate was identified as Exophiala sp.
partial β-tubulin gene (BT2). BLAST sequence homology searches revealed that the ITS sequence (568 bp) from our isolate showed a 98% similarity with strain ex-type of Exophiala psychrophila CBS 191.87 (accession number GenBank NR_145371.1). In order to confirm molecular identification at species level, phylogenetic relationships were performed with individual and combined genes using maximum-likelihood (ML) in MEGA v. 6.0 [dataset] [5]. For the ML analysis, nearest-neighbor interchange (NNI) was used as the heuristic method for tree inference; support for internal branches was assessed by 1000 ML sets of data. A bootstrap support (bs) ≥ 70 was considered significant. We included sequences of species into E. salmonis clade and E. brunnea CBS 587.66, was taken as outgroup.

On the basis of the macroscopic and microscopic features, and the results of concatenated analysis of the internal transcribed spacer (ITS) regions of rDNA and the partial β-tubulin gene (BT2), the isolate was identified as E. psychrophila into E. salmonis clade (Fig. 3).

DNA sequences obtained in this study were deposited in GenBank under accession numbers LS975365 for ITS, and LS975364 for β-tubulin.

3. Discussion

Exophiala psychrophila is one of the causative agents of chromoblastomycosis, in the literature cases are reported by Exophiala sp.; there are no case reports in which Exophiala psychrophila is included as an etiological agent; for which the use of molecular tools for its indispensable for identification.

The cases of chromoblastomycosis in Colombia reported in the literature are scarce, however is found a series of 10 cases of chromoblastomycosis due F. Pedrosoi, in which the efficacy of treatment with itraconazole was evaluated [dataset] [6] and a case auricular chromoblastomycosis caused by R. Aquaspersa that was also treated successfully with itraconazole [dataset] [7].

Histopathologically, chromoblastomycosis is characterized by the formation of granulomas that compromise the dermis in a variable way [dataset] [4] being these suppurative or tuberculoid; in addition to these, the mixed mycotic fungal granuloma is also described, which occurs exclusively in chromoblastomycosis and paracoccidioidomycosis. Although these findings guide the diagnosis, the
presence of mycotic structures and their phenotypic identification by molecular methods confirm the diagnosis [dataset] [8].

Individuals affected by chromoblastomycosis are usually men, farmers older than 30 years; in the case of our patient, is a 45-year-old farm woman. Due to her occupation, she acquired the disease through an injury in her foot, which had contact with soil, organic matter and the river side environment where the fungus was present [dataset] [8].

The therapeutic management is difficult, patients can often be refractory and tend to relapse. Among the drugs used are ketoconazole, voriconazole, 5-flucytosine, terbinafine, itraconazole, amphotericin B and posaconazole [dataset] [8]. It is worth mentioning that the recurrence rate is higher in cases where the antifungal therapy is less than six months.

This report describes a rare case of chromoblastomycosis caused by *E. psychrophila*. Currently, the *Exophiala* genus contains more than 40 species [dataset] [9], some of them have been reported as human opportunists. However other species are not related to a pathogenic role, showing a wide variety of virulence. Human is not the main host, animals have been described as first host; so that the lifestyle and environment are relevant risk factors for the human infection [dataset] [9].

The taxonomy of the *Exophiala psychrophila* species has not been sufficiently studied [dataset] [10], was derived from infected Atlantic salmon smolt (Salmo salar) in ocean water near Norway [dataset] [9,10], and previous and few reports of visceral infections caused by *Exophiala psychrophila* has been described [dataset] [10]. This is the first report related to a phaeohyphomycosis in an immunocompetent host.

However, members of *E. salmonis* [dataset] [11] clade had already been reported as human pathogens. The present case highlights the importance of the mycological diagnosis using molecular tools.**

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Conflicts of interest

None.

References


