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Sexual forms of yeasts in clinical samples

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Abstract

The sexual or teleomorphic state of yeasts has only been described in a few clinically involved species, mainly those of the *Saccharomycetaceae* family. With the aim of gathering information on their incidence in human pathology, a study has been made of a total of 2,135 strains isolated from clinical samples and cultivated in McClary agar. From these, 8 strains in teleomorphic state were identified: *Kluyveromyces marxianus* [1], *Pichia anomala* [2], *Pichia farinosa* [1], *Pichia membranaefaciens* [1] and *Saccharomyces cerevisiae* [3]. The two strains of *P. anomala* were responsible for fungemia; *K. marxianus* and the two strains of *S. cerevisiae* produced vaginitis; the other strains were oral cavity colonizers.

Key words: Yeasts, Ascospores, *Kluyveromyces*, *Pichia*, *Saccharomyces*

Introduction

It is possible that all the yeast-like fungi possess a sexual or teleomorphic state, although this has only been described in a small number of species involved in human pathology. Most of the known teleomorphic human pathogens are found in the *Ascomycotina* subdivision, *Hemiascomycetes* class, *Saccharomycetaceae* family. It is exceptional for them to be discovered in clinical samples, either because their incidence is perhaps very low, or because the existence of ascospores whose sexual state is identified does not figure in routine research or analysis. From a full review of the literature, only a very few cases have been found of human infection reulting from *Kluyveromyces marxianus* (*K. fragilis*, *K. lactis*) [1-3] and *Pichia angusta* (*Hansenula polymorpha*) [4], some thirty cases attributed to *Pichia anomala* (*Hansenula anomala*) [5–22] and to *Saccharomyces cerevisiae* [23–42] and one isolated case of infection from *Yarrowia lipolytica* [43].

Given this relative lack of information on the true clinical incidence of yeast teleomorphs, we thought it would be interesting to investigate the presence of ascomycetes in a large number of samples analyzed in a regular diagnostic routine, with the aim of quantifying the extent of their participation in infectious processes.

Material and methods

A total of 61,212 samples obtained from patients with various pathologies attended in the University Hospital 'Puerta del Mar' of Cádiz, Spain, were studied; from these, 2,135 different strains of yeast were isolated and identified by means of the study of their morphological, physiological, biochemical and nutritional characteristics, using conventional methods and the commercial system 'ATB 32' (Bio Mérieux, France).

Ascosporogenesis was tested on the medium of McClary agar with acetate [44], inoculated from a 48-hr culture in Sabouraud dextrose agar and incubated for 21 days at 25 °C. Presence of ascospores was verified at intervals of 7, 14 and 21 days by microscopic observation with lactophenol blue and by means of Kinyoun staining.

Results

A total of 8 yeast strains in teleomorphic state, comprising 3 different genera and 5 different species: *Kluyveromyces marxianus* (1 strain); *Pichia anomala* (2 strains); *Pichia farinosa* (1 strain); *Pichia membranaefaciens* (1 strain); and *Saccharomyces cerevisi-*

Table 1. Yeast strains in teleomorphic state isolated from clinical samples

Patients	Sex	Age	Yeasts	Samples	Clinical signification
1	Female	33	*Kluyveromyces marxianus*	Vaginal exudate	Vaginitis
2	Male	5 days	*Pichia anomala*	Blood	Fungemia
3	Male	51	*Pichia anomala*	Blood	Fungemia
4	Male	23	*Pichia farinosa*	Oral cavity	Colonization
5	Male	27	*Pichia membranaefaciens*	Sputum	Colonization
6	Female	32	*Saccharomyces cerevisiae*	Vaginal exudate	Vaginitis
7	Female	28	*Saccharomyces cerevisiae*	Vaginal exudate	Vaginitis
8	Female	56	*Saccharomyces cerevisiae*	Oral cavity	Colonization

ae (3 strains) (Table 1). The incidence of these 8 strains in the total of all yeasts was 0. 37% and, in relation to the total samples analyzed, 0. 01%.

Under the microscope, *K. marxianus* presented evanescent ascos containing from 2 to 4 kidney – or crescent – shaped spores, similar to orange segments; *P. anomala* presented evanescent ascos with up to 4 spores, spheroid and hat-shaped: *P. farinosa* presented very few persistent ascos with 1 or 2 round spores in their interior; *P. membranaefaciens* evanescent ascos with 2 to 4 small round spores; *S. cerevisiae*, persistent ascos containing 1 to 12 large round and oval spores.

K. marxianus was isolated from the vaginal exudate of a 33-year-old woman with clear signs of vaginitis: leucorrea, pruritis and vulvovaginal erythema. One strain of *P. anomala* was obtained from the blood of a 26-week gestation neonate, who 5 days after birth presented outbreaks of apnea, anemia with thrombocytopenia and signs of sepsis related to the parental nutrition catheter, from which the same yeast was also separately isolated. The other strain of *P. anomala* was isolated from the blood and aorto-bifemoral prosthesis of a 51-year-old patient who had previously undergone surgical intervention for the insertion of the prosthesis and who later suffered contamination of the bile. *P. farinosa* was found forming part of the oral cavity flora, together with *Candida krusei*, in an AIDS-diagnosed patient being treated with fluconazol. *P. membranaefaciens* was isolated, together with *Candida parapsilosis*, from the sputum of a patient infected with human immunodeficiency virus (HIV). The three strains of *S. cerevisiae* were obtained, respectively, from the vaginal exudate of a 32-year-old woman with recurrent candidiasic vaginitis, associated with chronic symptoms of dispareunia and hyperplasia, from the vaginal exudate of a woman 18 weeks pregnant, with moderate signs of vaginitis, and from oral cavity exudate of a woman suffering oseal necrosis and undergoing antibiotherapy and radiotherapy.

Discussion

The involvement of ascomycetes in human pathology is infrequent, and only a few species have been described, as producers of infections, particularly in pediatric and immunocompromised patients and those with serious illnesses.

Kluyveromyces marxianus, the teleomorph of *Candida kefyr*, has been referred to as an opportunistic pathogen on three occasions [1–3]; in our case, it was considered the cause of vaginitis, given the evident symptoms in the absence of other possible microbial etiology.

Pichia anomala (*Hansenula anomala*), the teleomorphic state of *Candida pelliculosa*, has been associated with interstitial pneumonia in children [5–7], with infection of the urinary tract [15], with infection of oral mucous [8], peritonitis [20], meningitis [10], endocarditis [11] and, above all, fungemia [9–14, 16–22]. The two cases of sepsis reported here presented a fatal evolution, despite the very limited pathogenic property attributed to this species.

Pichia farinosa does not present a known sexual form nor has it been described as a pathogen; it has been found in the oral mucous of cancer patients receiving radiotherapy [45], the same localization from which it was isolated in our AIDS patient.

Pichia membranaefaciens is the teleomorph of *Candida valida*; we are not aware of any previous isolation from clinical samples; our discovery in the sputum of an HIV infected patient was not related to respiratory infection.

Saccharomyces cerevisiae, whose anamorphic state is *Candida robusta*, has been implicated in isolated cases of respiratory infection [23, 31, 36–38], infection of the urinary tract [31], vulvovaginitis [25, 35, 40, 42], peritonitis [29, 30], empyema [41], endocarditis [24, 26] and fungemia [24, 26–28, 32–34, 37, 39]. Here we present two new cases of vaginitis and one of possible oral infection in an immunocompromised patient.

In our experience, the isolation of yeasts in teleomorphic state in clinical samples is a fairly rare occurrence. The results of this study indicate an incidence of only 0.4% of all the yeasts found present in these samples.

However, in spite of such low incidence, the production of ascospores by a specific strain confirms that we are dealing with a species with a different growth cycle and, from the taxonomic viewpoint, different from that corresponding to the anamorphic state. The close connection observed between these strains and serious infections or a specific type of patient, generally immunocompromised, suggests the advisability of correctly identifying the ascomycetes, particularly since the procedure for ascospore detection is simple and within the scope of any laboratory.

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