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Genetic Diversity and Taxonomic Implications of *Diutina mesorugosa*: A *review*

Diversidade Genética e Implicações Taxonômicas de *Diutina mesorugosa*: Uma *revisão*

Diversidad Genética e Implicaciones Taxonómicas de Diutina mesorugosa: Una revisión

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Abstract
Diutina mesorugosa is a yeast emerging in hospital environments and has been isolated from different clinical samples in different locations around the World, although atypical as an etiological agent of human infection. Previous phylogenetic analyses assigned the species as *Candida mesorugosa*, but this yeast has been reclassified as *D. mesorugosa*. It is known that different species belonging to the same genus such as *D. rugosa*, *D. pseudorugosa*, and *D. catelunata* have also been isolated from clinical samples, and the *D. rugosa* species has been identified as an emerging opportunistic pathogen. These species have also been reclassified recently. The objective was to perform a literature review on the taxonomic aspects of the yeast *D. mesorugosa* to deepen the knowledge of the taxonomic classification and systematics of this species within the genus Diutina.
Keywords: *Yeast; Diutina mesorugosa;* Taxonomy.

Resumo
Diutina mesorugosa é uma levedura que está emergindo em ambientes hospitalares, e tem sido isolada de diferentes amostras clínicas em diversas localidades ao redor do mundo, ainda que atípica como agente etiológico de infecção humana. Análises filogenéticas anteriores atribuíram a espécie como *Candida mesorugosa*, mas, atualmente, esta levedura foi reclassificada como *D. mesorugosa*. Sabe-se que diferentes espécies pertencentes ao mesmo gênero como *D. rugosa, D. pseudorugosa, e D. catelunata* também já foram isoladas de amostras clínicas, e a espécie *D. rugosa* foi apontada como um patógeno oportunista emergente. Essas espécies também foram reclassificadas recentemente. O objetivo foi realizar uma revisão bibliográfica sobre os aspectos taxonômicos da levedura D. mesorugosa para aprofundar o conhecimento da classificação taxonômica e sistemática desta espécie dentro do gênero Diutina.
Palavras-chave: Levedura; *Diutina mesorugora;* Taxonomia.

Resumen
Diutina mesorugosa es una levadura que está emergiendo en ambientes hospitalarios y ha sido aislada de diversas muestras clínicas en varias localidades alrededor del mundo, aunque es atípica como agente etiológico de infección humana. Análisis filogenéticos previos asignaron a la especie como *Candida mesorugosa*, pero actualmente esta levadura ha sido reclasificada como *D. mesorugosa*. Se sabe que diferentes especies pertenecientes al mismo género, como *D. rugosa*, *D. pseudorugosa* y *D. catelunata*, también han sido aisladas de muestras clínicas, y la especie *D. rugosa* ha sido señalada como un patógeno oportunista emergente. Estas especies también fueron reclasificadas recientemente. El objetivo fue realizar una revisión bibliográfica sobre los aspectos taxonómicos de la levadura *D. mesorugosa* para profundizar el conocimiento de la clasificación taxonómica y sistemática de esta especie dentro del género Diutina.
Palabras clave: Levadura; *Diutina mesorugosa*; Taxonomía.

1. Introduction

The yeast Diutina mesorugosa has been associated as an etiological agent of human infections, isolated from clinical samples of hospitalized patients, in different locations, in more than one continent (Chaves et al., 2013; Adjapong et al., 2016; Mathur et al., 2018; Owoicho et al., 2020). Recently, *D. mesorugosa* was observed as an agent of candidemia in a tertiary

hospital, Trauma Center, in Delhi, India (Mathur et al., 2018). D. (Candida) mesorugosa was isolated by Owoicho et al. (2020) from a urogenital sample of a patient suffering from postpartum vulvovaginitis in a tertiary hospital in southwestern Nigeria, and the species was identified by sequencing the internal transcribed spacer region (ITS1-5.8S-ITS2) of ribosomal DNA. In 2016, Adjapong and collaborators isolated D. (Candida) mesorugosa from urine samples collected from patients with urinary tract infections in Ghana, Africa. In Brazil, strains of *D. mesorugosa* were obtained from clinical samples of human blood, human rectal swab and pericatheter swab, in a tertiary hospital, and were initially identified as a species similar to Candida (Diutina) rugosa, and later, genotypic analyses confirmed that it was a new species, which was named Candida mesorugosa, currently reclassified as *D. mesorugosa* (Chaves et al., 2013; Khun-namwong et al., 2015).

Information on infections caused by *D. mesorugosa* is still scarce, however, given the evidence provided by the scientific community regarding the occurrence of this microorganism associated with different types of clinical samples, it is understood that *D. mesorugosa* is potentially relevant yeast in hospital environments (Mathur et al., 2018; Owoicho et al., 2020). It is known that different species belonging to the genus Diutina, such as *D. rugosa*, D. pseudorugosa, and D. catenulata, have also been isolated from clinical samples (Li et al. 2006; Ming et al. 2019). *D. rugosa* has been identified as an emerging opportunistic pathogen that can occur in fungemia clusters, infecting critically ill patients and immunocompromised individuals who have undergone invasive medical practices, such as catheter use or previous surgery (Pfaller et al., 2010; Singh et al., 2011; Ming et al., 2019). These species were also recently reclassified to the genus Diutina (Khunnamwong et al., 2015).

Due to the reclassification and molecular characterization of the species *D. mesorugosa* within the genus Diutina, there is a demand for consistent data on the putative clinical peculiarities, antifungal susceptibility, ecology, pathogenicity and biotechnological potential of the species belonging to this genus, especially the species *D. mesorugosa*. In this context, the objective was to perform a literature review on the taxonomic aspects of the yeast *D. mesorugosa* to deepen the knowledge of the taxonomic classification and systematics of this species within the genus Diutina.

2. Methodology

Qualitative research was carried out (Pereira et al., 2018) based on a literature review, as a form of research, to obtain information and analyze them (Snyder, 2019).

This study is a narrative literature review (Rother, 2007, Cavalcante & Oliveira, 2020; Casarin et al., 2020), comprising a broad analysis of published references on the species *Diutina mesorugosa* in various occurrences and environments. Searches were carried out in the databases Pubmed (National Center for Biotechnology Information), Scielo (Scientific Electronic Library Online), Medline (Medical Literature Analysis and Retrieval System Online), and Google Scholar, under the search criteria *Candida rugosa*, *Candida mesorugosa*, *Diutina rugosa* and *Diutina mesorugosa.*

3. Results and Discussion

3.1 Ecology

Yeasts are unicellular fungi that reproduce mainly by budding or fission. They are ubiquitous in their distribution and can be found in the most varied environments and substrates (Gomes et al., 2011, Péter et al., 2017; Buzzini et al., 2017). The ecology, species delineation and phylogenetic placement of the members of the genus Diutina are still little known, given the small number of species in this group (Khunnamwong et al., 2015). However, in addition to reports of isolation of these microorganisms from clinical samples such as blood, lesions, feces, and sputum, there are records of isolation from rice leaf tissue, bovine food products such as milk and cheese (Li et al., 2006; Paredes et al., 2012; Chaves et al., 2013; Khunnamwong

et al., 2015; Dion and Dukes 1982; Álvarez-Martín et al., 2007; Şeker et al., 2010), and Arctic ice (Butinar et al., 2011). Furthermore, *D. mesorugosa* was recovered from water samples from an oil refinery effluent dike, an environment impacted by waste from crude oil refining (Peixoto, 2017).

3.2 Taxonomy

Fungal taxonomy has shown significant advances through molecular identification techniques and has led to the taxonomic reorganization of many fungal taxa (Montoya et al., 2019). Molecular techniques, combined with sequencing as a means of identifying yeasts, and more specifically, the establishment of ITS and D1-D2 sequence analysis as a phylogenetic tool, have allowed the differentiation and regrouping of several species, including *D. mesorugosa* (Montoya et al., 2019; Khunnamwong et al., 2015).

Diutina mesorugosa was originally introduced as *Candida mesorugosa* by Chaves et al. (2013) in Brazil. It is part of a species complex comprising four taxa previously classified as *Candida rugosa, Candida pseudorugosa, Candida neorugosa, and Candida mesorugosa* (Paredes et al., 2012; Owoicho et al., 2020). The differentiation of the phenotypically indistinguishable species that formed what was then considered the *C. rugosa* complex can be characterized through molecular analyses (Montoya et al., 2019). Currently, after phylogenetic and molecular analyses, these taxa have been reclassified to the genus Diutina gen. nov., proposed with the aim of accommodating all members of the clade, which includes the species *Diutina siamensis fa sp. nov., Candida catenulata, Candida mesorugosa, Candida neorugosa, Candida pseudorugosa, Candida rugosa, Candida ranongensis,* and *Candida scorzettiae* (Khunnamwong et al., 2015, Ming et al., 2019). Currently, phylogenetically, the genus Diutina is in the order Saccharomycetales, subphylum Saccharomycotina, phylum Ascomycota (Khunnamwong et al., 2015).

The phenotypic characterization of yeasts is performed through the analysis of their micromorphological characteristics and biochemical profile. Although phenotypic analysis is time-consuming and does not guarantee an accurate differentiation between yeast genera, it is essential in the identification of these microorganisms and complements molecular taxonomy and vice versa (Montoya et al., 2019; Ming et al., 2019).

Species belonging to the genus Diutina have ovoid to ellipsoidal cells, pseudohyphae, with growth by multilateral budding in a temperature range of 28 and 33 °C, and the ability to develop in the absence of amino acids, but not in the absence of vitamins (Khunnamwong et al., 2015, Chaves et al., 2013). The species *D. mesorugosa* is characterized by its smooth, cream-colored, opaque and soft colonies. They form abundant blastospores and pseudohyphae when grown on Cornmeal Agar (CMA) (Chaves et al., 2013). Species of the genus Diutina are capable of using ethylamine, lysine and cadaverine as the sole source of nitrogen. They can be differentiated according to their ability to ferment sugars such as glucose, lactose, galactose, trehalose, maltose, sucrose, ethanol, glucitol, succinic acid, D-glucose, D-sorbitol, D-xylose, D-mannitol, lactate, glycine, N-acetylglucosamine and hexadecane (Khunnamwong et al., 2015, Chaves et al., 2013).

Regarding susceptibility to antifungals, the species *D. rugosa*, *D. mesorugosa* and *D. pseudorugosa* when tested against four antifungal agents in a study carried out by Ming et al. (2019), showed sensitivity to anphotericin B (MIC 0.5−1 μg/ml), fluconazole (MIC 0.5−1 μg/ml) and 5-flucytosine (MIC 0.5−1 μg/ml). While *D. neorugosa* isolates were dose-dependently sensitive to itraconazole (MIC 0.25 μg/ml) and sensitive to amphotericin B (MIC 0.5-1 μg/ml), fluconazole (MIC 0.5-1 μg/ml) and 5-flucytosine (MIC 0.125 μg/ml) (Ming et al., 2019). In a 10-year multicenter study conducted by Pfaller et al. (2010), *D. rugosa* stands out along with other species due to its decreased susceptibility to azoles and other antifungal agents, and as a cause of invasive candidiasis. The susceptibility profile exhibited by *D. rugosa* against the tested antifungal agents showed that voriconazole (69.3% S) was more active than fluconazole (49.9% S), which showed decreased susceptibility (Pfaller et al., 2010). Data obtained by Montoya et al. (2019), when performing in vitro antifungal susceptibility

testing with nine *D. mesorugosa* isolates using the microdilution method, showed that all tested isolates presented MICs 1 μg/mL for amphotericin B, eight of nine (88.8%) of the isolates were susceptible to fluconazole (MICs 2 μg/mL), and all isolates were resistant to anidulafungin and caspofungin (MICs 1 μg/mL).

3.3 Virulence Factors and Pathogenicity

Yeasts with greater potential for morbidity are more studied when compared to those rarely isolated in the clinical context, creating a gap in information on the virulence and antifungal susceptibility patterns of these microorganisms, and a growing demand for new data (Montoya et al., 2019). Regarding species of the genus Diutina, although they may play an important role as an etiological agent in fungal diseases and opportunistic infections, little is known about their pathogenicity and virulence determinants (Montoya et al., 2019). However, the species *D. rugosa* has been reported as a causative agent of veterinary infections (Moretti et al., 2000; Crawshaw et al,. 2005; Scaccabarozzi et al., 2011), thus generating great concern, given the possible negative impact on industry and the economy (Mixão et al., 2019). This species has been considered an emerging fungal pathogen and identified as the etiological agent of several clinical infections (Colombo et al., 2003; Pfaller et al., 2006; Minces et al., 2009; Pfaller et al., 2010). Montoya et al. (2019) evaluated the in vitro virulence determinants and comparative pathogenicity of nine clinical isolates of *Diutina mesorugosa* using a *Galleria mellonella* survival model and detected positive aspartyl protease activity in all strains evaluated. In addition, esterase activity was detected in seven of the nine isolates. The authors also evaluated DNase and hemolysin activities, which were evident in only two of the isolates studied. Regarding the ability to produce biofilm, all isolates formed biofilm after 72 h of incubation. Two of the strains studied (HPM309 and H259) were able to generate an acute infection and exhibited the highest virulence, while the isolates *D. mesorugosa* 99-480 and DM17 proved to be the least virulent strains.

3.4 Biotechnological Applications

Species belonging to the genus Diutina have been widely studied due to their biotechnological properties (Domínguez et al. 2006; Chaves et al. 2013; Peixoto, 2017). *D. mesorugosa* was cited as a species capable of degrading polycyclic aromatic hydrocarbons (PAHs) (Peixoto, 2017). The species *D. rugosa* also presents several characteristics that make it useful yeast to produce extracellular lipases, which have been widely used in industry, the production of fatty acids, the synthesis of various esters, and the kinetic resolution of racemic mixtures (Domínguez et al., 2006, Hsu, et al., 2008). In addition, *D. rugosa* is also known for its potential for biodegradation of indigo dye (Bankole et al., 2017).

4. Conclusion

Species belonging to the genus Diutina are described with limited data related to their phenotypic, physiological, and biochemical characteristics. Distinguishing between species depends on molecular methods due to the phenotypic similarities between them, especially when we refer to the differentiation of the species *D. rugosa* and *D. mesorugosa*, thus confirming the importance of molecular tools in the taxonomy of phenotypically indistinguishable species within the *D. rugosa* species complex. The species *D. mesorugosa* is still little studied, and the role played by this phylogenetically unique species within the genus Diutina is not known for sure, and it has been isolated infrequently from non-clinical substrates. Thus, given its clinical and biotechnological importance, and recent reclassification, more comprehensive and in-depth studies on the species and their importance in natural and hospital environments are necessary.

Conflicts of interest

The author declares that there are no conflicts of interest. The author is solely responsible for the content and writing of this article.

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