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Ascospore release from bottle-shaped asci in *Dipodascus albidus*

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Abstract

Yeasts utilize different mechanisms to release ascospores of different lengths from bottle-shaped asci. Using electron microscopy, confocal laser scanning microscopy, gas chromatography–mass spectrometry and digital live imaging, the individual release of oval ascospores from tight-fitting narrow bottle-necks, is reported in the yeast *Dipodascus albidus*. These ascospores are surrounded by compressible, oxylipin-coated sheaths enabling ascospores to slide past each other when forced by turgor pressure and by possible sheath contractions towards the narrowing ascus-neck. In this paper, the release mechanisms of ascospores of various lengths from bottle-shaped asci and produced by different yeasts are compared. We suggest that different release mechanisms, utilizing compressible sheaths or geared-alignment, have possibly evolved to compensate for variation in ascospore length. Alternatively, sheaths and ridges might be two evolutionary solutions to the same biomechanical problem, i.e. to release ascospores irrespective of length from bottle-shaped asci.

Keywords: Alignment; Ascospore; *Dipodascus albidus*; 3-Hydroxy oxylipin; Release mechanism; Sheath

1. Introduction

In 1991, we discovered the first aspirin-sensitive oxylipins in yeasts [1,2]. Since then studies by various research groups have demonstrated the ubiquitous nature of these compounds in yeasts and their importance as target to control fungal infections [1,3–6]. We recently exposed another feature of fungal oxylipins [7]. In some yeasts oxylipins, such as 3-hydroxy oxylipins, were found to act as lubricants during ascospore release from enclosed asci [7]. This research opened new views on ascospore movement in micron-space, which may find application in nano-, aero- and hydro-technologies [7].

Microscopic studies revealed that representatives of the yeast genus *Dipodascopsis* and some *Dipodascus* species produce bottle-shaped asci with a broad base and narrow neck, containing ascospores of various shapes (round, oval, or elongated) with surface ornamentations (compressible sheaths or surface ridges linked in gear-like manner) [7,8]. Each yeast species produces only one kind of ascospore structure. These morphological differences may influence the type of release mechanism used by a particular species to force ascospores, probably by turgor pressure, through tight-fitting ascus openings without blocking the ascus tip [7–9]. This is in

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accordance with the literature where it has been reported that many ascomycetous fungi release their ascospores forcibly from asci through osmotic or turgor pressure [10].

In *Dipodascus aggregatus*, round to oval-shaped ascospores are enveloped in oxylipin-coated compressible sheaths [7]. These sheaths enable ascospores to slide past each other when reaching the narrowing ascus neck. However, more elongated ellipsoidal to reniform ascospores of *Dipodascopsis uninucleata* var. *uninucleata* are released differently [7–9]. Here, the elongated ascospores remain aligned within the bottle-shaped ascus before release. Otherwise, we believe they might turn sideways, thereby blocking the ascus-neck and eventually inhibiting individual ascospore release. These ascospores do not contain sheaths, but are linked by means of interlocked ridges on the surfaces of neighboring ascospores, thereby keeping them aligned while being pushed towards the ascus-tip. It is proposed that 3-hydroxy oxylipins also assist in this release mechanism by acting as a lubricant between ascospores [7–9,11].

This study explores the secret behind the release mechanism of oval-shaped ascospores from bottle-shaped asci in the yeast *Dipodascus albidus*. These findings are compared with possible mechanics involved in effective release of ascospores of different lengths from similarly shaped asci.

2. Materials and methods

2.1. Strains and cultivation

Dipodascus albidus UOFS Y-1445T, *Dipodascus aggregatus* UOFS Y-1358 and *Dipodascopsis uninucleata* var. *uninucleata* UOFS Y-128 were used in this study.

These strains are held at the University of the Free State, Bloemfontein, South Africa. The yeasts were streaked on yeast malt agar [12] and cultivated at room temperature for 2–10 days until sporulation was observed. All experiments were performed at least in duplicate.

2.2. Asci and ascospore measurements

The dimensions (diameter and length) of one hundred ascospores within various asci of *Dipodascus albidus*, *Dipodascus aggregatus* and *Dipodascopsis uninucleata* var. *uninucleata* were measured using a micrometer fitted to a light microscope. Subsequently, the ratios of ascospore diameter:length were calculated. Since the release mechanisms of *Dipodascus aggregatus* and *Dipodascopsis uninucleata* var. *uninucleata* have been studied previously [7], only the ascospore release mechanism of *Dipodascus albidus* was further investigated.

2.3. Ascospore release studies in Dipodascus albidus

To illustrate release of individual ascospores from asci tips, asci with ascospores (unstained and stained according to [13]) were studied by light microscopy. Photographs and a movie showing active ascospore release were taken using an Axioplan light microscope (Zeiss, Göttingen, Germany) coupled to a Colorview Soft Imaging System (Münster, Germany).

2.4. Immunofluorescence microscopy of Dipodascus albidus

Antibodies against chemically synthesized 3*R*-hydroxy-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid [14] were raised in rabbits and then characterized by determining their sensitivity, titer and specificity [11].

Immunofluorescence microscopy was performed as described [11]. Cells (5 $mg l^{-1}$) were suspended in 100 ml phosphate buffer solution and centrifuged for 10 min to remove debris and agar. Next, 30 μl of primary antibody was added to the cells and incubated for 60 min to allow sufficient binding to the oxylipins. After washing with phosphate buffer solution, fluorescein isothiocyanate (FITC) secondary antibodies (Sigma, St. Louis, MO, USA) were added and incubated in the dark for 30 min to allow sufficient binding to the primary antibody. To ensure that aggregated ascospore structure was maintained, antibody, fluorescence and wash treatments were performed in 2-ml plastic tubes. The cells were fixed on a microscope slide and photographed using a Nikon 2000 Confocal Laser Scanning Microscope (Nikon, Tokyo, Japan).

2.5. Orange-G staining

Cells from a 10-day-old culture of *Dipodascus albidus* were stained with 1% Orange G (Molecular Probes, Eugene, OR, USA) for 5 min at room temperature. After staining, cells were rinsed twice with distilled water. Micrographs were obtained using a Nikon 2000 Confocal Laser Scanning Microscope.

2.6. Electron microscopy

Cells from a 10-day-old culture of *Dipodascus albidus* were chemically fixed, using 3% glutaraldehyde (Merck, Darmstadt, Germany) and 1% osmium tetroxide (Merck) [15]. These cells were dehydrated by a graded ethanol series, followed by drying using a critical-point dryer. The specimen was made electron-conductive by mounting the sample on a stub and coating it with gold. Scanning electron micrographs were taken with a Jeol 6400 WINSEM (Jeol, Tokyo, Japan).