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Systematic and Applied Microbiology 29 (2006) 446–449
SYSTEMATIC AND APPLIED MICROBIOLOGY
www.elsevier.de/syapm

SHORT COMMUNICATION

Mapping the distribution of 3-hydroxy oxylipins in the ascomycetous yeast *Saturnispora saitoi*

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Received 1 August 2005

Abstract

The distribution of 3-hydroxy oxylipins in *Saturnispora saitoi* was mapped using immunofluorescence microscopy. Fluorescence was observed on aggregating ascospores, indicating the presence of 3-hydroxy oxylipins on the surface or between ascospores. The oxylipin was identified as 3-hydroxy 9:1 using gas chromatography mass spectrometry. Furthermore, ultrastructural studies using scanning and transmission electron microscopy on ascospores revealed a clear equatorial ledge surrounding oval-shaped ascospores.

Keywords: Ascospores; Electron microscopy; 3-Hydroxy oxylipins; Immunofluorescence; *Saturnispora saitoi*; Ultrastructure; Yeast

Introduction

The distribution of 3-hydroxy oxylipins in yeast was previously reported using polyclonal antibodies as well as gas chromatography mass spectrometry analysis [5,13]. Consequently, these compounds were found to be associated with aggregating sexual as well as asexual vegetative cells in the yeasts *Candida albicans* [1,2], *Dipodascopsis uninucleata* var. *uninucleata* [4], Dipodascaceae [11], Lipomyces [12], *Saccharomyces cerevisiae* [6], *Saccharomycopsis malanga* [9] and others [8,10]. Since 3-hydroxy oxylipins show potent biological activities in medical studies [3] and importance in ascospore formation and movement in micron-space, bioprospecting studies are undertaken to expose rich sources of these compounds for eventual biotechnological production and decoding of function.

In this study, we map the distribution of 3-hydroxy oxylipins in the ascomycetous yeast *Saturnispora saitoi* while ascospore ultrastructure is elucidated.

Materials and Methods

Strain used and cultivation

Saturnispora saitoi UOFS Y-1243, held at the University of the Free State, Bloemfontein, South Africa, was used throughout the study and cultivated on YM (Yeast-malt) agar slants [15], transferred to YM agar medium and incubated at 25 °C until sexual stage was

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doi:10.1016/j.syapm.2005.11.008

reached. Upon sexual stage, cells were subjected to immunofluorescence, and electron microscopic studies while cell extracts were subjected to gas chromatography mass spectrometry.

Immunofluorescence microscopy

The preparation and characterisation of antibodies against these oxylipins were according to Kock et al. [5]. Antibody and fluorescine labelling as well as eventual immunofluorescence microscopy were performed as described [5].

Electron microscopy

This was performed according to Van Wyk and Wingfield [14]. In short, cells were chemically fixed in glutaraldehyde (over night) and osmium tetroxide (two hours) at room temperature. Electron micrographs were taken with a Joel 6400 WINSEM (Japan) and a Philips CM 100 (The Netherlands) TEM.

Gas chromatography mass spectrometry (GC MS)

Extraction of oxylipins followed by methylation, silylation and GC MS analysis was performed according to Sebolai et al. [9].

Chemicals used

All chemicals used were of highest purity grade and obtained from reputable dealers.

Results and discussion

When subjecting cells of *Saturnispora saitoi*, treated with fluorescine-labelled antibodies (i.e. fluorescine anti rabbit IgG), to immunofluorescence microscopy, mainly the ascospores fluoresced (Fig. 1a and b). By closer inspection we found that these compounds are associated with surfaces of ascospores (Fig. 1b). The presence of these oxylipins was confirmed by gas chromatography mass spectrometry. Here a characteristic mass ion at 175 (indicating hydroxyl group at position 3 of the fatty acid) was identified [13] thereby indicating the presence of a 3-hydroxy oxylipin. The structure of this compound was found to be 3-hydroxy 9:1 with the following characteristic mass ions: 175 [$CH_3O(CO)–CH_2–CHO–TMSi$] and 243 [M^+-15] (Fig. 2).

Since 3-hydroxy oxylipins are associated with the surfaces of these ascospores, we decided to study their ultrastructure and especially the nano-scale ornamentations, which are covered with oxylipins. Consequently, we found that oxylipins are attached to cell walls of oval shaped ascospores, each surrounded by a sub-equatorial ledge (Figs. 3 and 4). This is in accordance to literature where similar observations were made regarding ascospore morphology of the closely related *Saturnispora ahearnii* using scanning electron microscopy [7].

The function (s) of these oxylipins in ascospore formation, aggregation and release from asci are now under investigation.

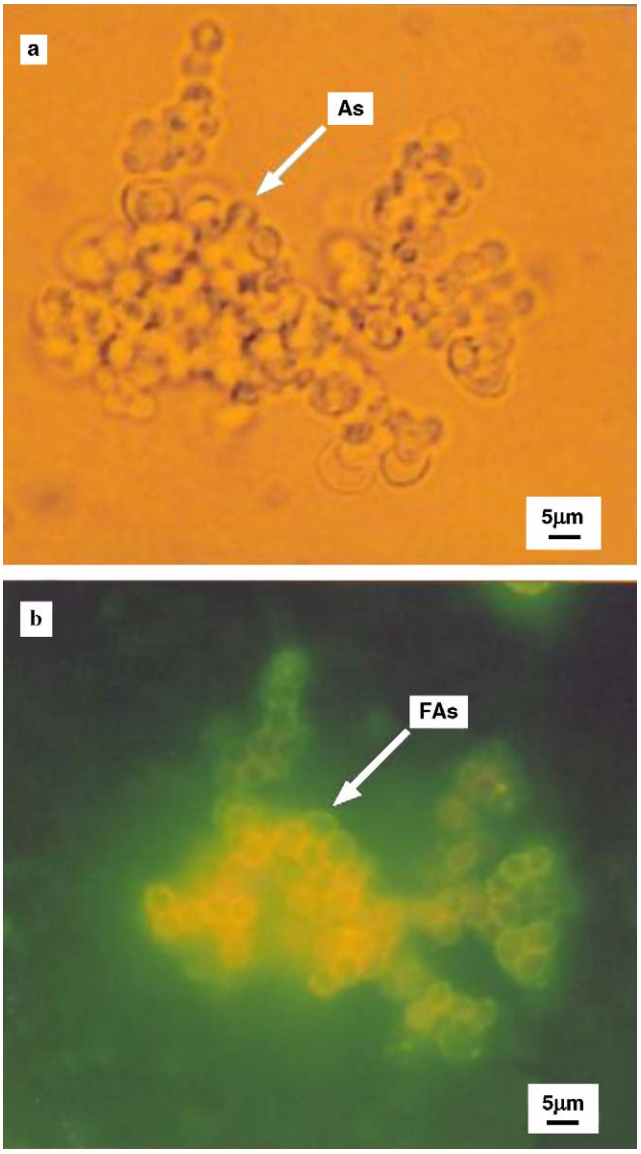

Fig. 1. Light and immunofluorescence micrographs of *Saturnispora saitoi* showing aggregating saturn-shaped ascospores: (a) light micrograph showing saturn-shaped ascospores (As) aggregating after released from the ascus and (b) immunofluorescence micrograph showing fluorescing ascospores (FAs) after treatment with an antibody specific to 3-hydroxy oxylipins.