Exploring the mechanism of action of two tick derived antimicrobial peptides, Os and Os-C, against planktonic *Candida albicans*

Mandelie van der Walta,*, Dalton Mollera,*, Helena Tauteb, Megan J. Besterb, Anabella R. M. Gaspara

University of Pretoria, South Africa

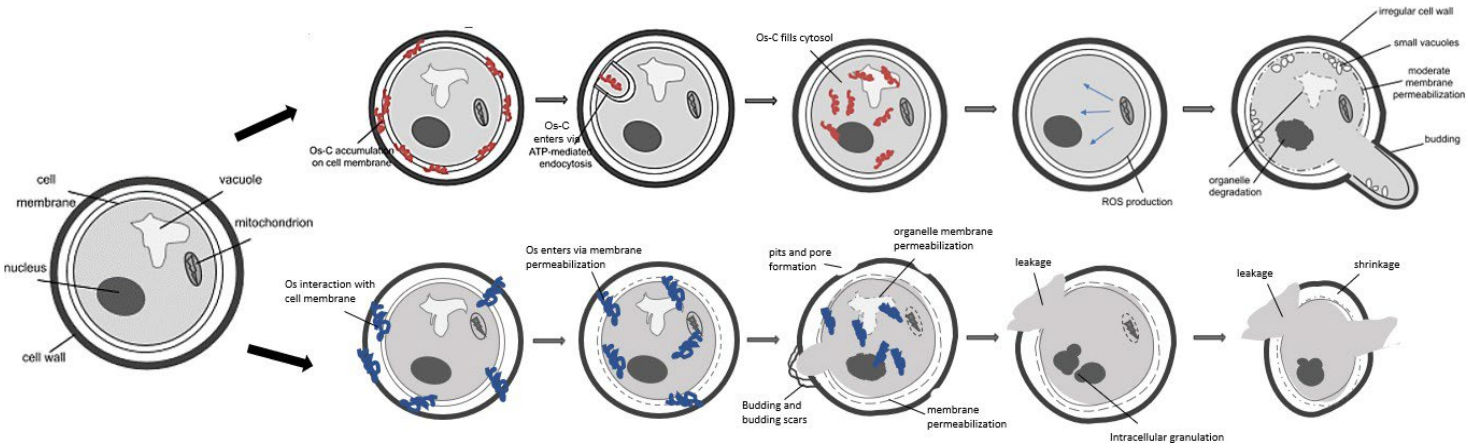

Figure 1: A proposed mode of action of Os and Os-C against planktonic C. albicans cells. *Os-C (red) crosses the cell wall, accumulates on the cell membrane, translocates via ATP-mediated endocytosis, fills the entire intracellular space, induces ROS-production, causes collapse in cell structures, membrane permeabilization, organelle degradation and bud and hyphal cell formation. Os (blue) crosses the cell wall, interacts with the membrane, enters the cytosol through membrane permeabilization, forms pits and pores in the cell surface, permeabilizes intracellular organelles, forms buds and bud scars leading to cytosol leakage and cell shrivelling.*

Fungal pathogen resistance has increased rapidly, creating an urgent need for new antifungal drugs [1]. Antimicrobial peptides (AMPs) are promising candidates due to their broad-spectrum of cellular targets but little is known about their mechanism of action [2]. This study further investigated the mechanism of action of two tick derived AMPs, Os and Os-C [3,4], against planktonic *C. albicans*. For rapid screening of salt-sensitive AMPs a modified microbroth dilution assay was developed and IC_{50} of 1.163 ± 0.116 µM and 4.69 ± 0.18 µM were determined, for Os and Os-C respectively. Electron microscopy revealed that sub-MIC concentrations of Os caused drastic and non-specific effects inducing pits on the cell surface, pore formation in the cell membrane, as well as increased budding scars, while Os-C caused depression in cell surfaces, cytoplasmic retraction, formation of buds, small vacuoles adjacent to the cell membrane and intracellular granulation. Although cell wall polysaccharide binding had previously been reported, isothermal titration calorimetry and serial dilution assays indicated that neither peptide binds the cell wall polysaccharides, laminarin or mannan, at low, physiological concentrations. Membrane depolarisation evaluated with the probe, bis-(1,3- dibutylbarbituric acid) trimethine oxonol (DiBAC4(3)) seemed not to be the primary mode of action of either peptide, but rather the aftermath of membrane permeabilization. Flow cytometry and confocal microscopy showed that both peptides entered the cells with Os localizing within the cell membrane and nucleus at lower concentrations and Os-C accumulating in the cytosol. At higher concentrations only Os showed significant vacuole degradation. Differences in ultrastructural effects, peptide uptake and intracellular targets between Os and Os-C suggest that these AMPs have distinct modes of action.

Keywords: Fungal pathogen resistance, antimicrobial peptides, mode of action, tick, salt-sensitive, *Candida albicans*

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