Volatiles produced by *Candida* Spp

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Introduction

Candida albicans is a fungus capable of causing denture stomatitis, the most common form of oral candidiasis, present in up to 60% of denture wearers, affecting predominantly patients over 65 years old (Daniluk et al, 2006). Symptoms include mucosal bleeding, swelling, burning and other painful sensations, halitosis, unpleasant taste and dryness in the mouth (Dorocka-Bobkowska et al, 2010). Like most other fungi, *C. albicans* is capable of producing microbial volatile organic compounds (MVOCs), with over 150 described in the literature

It is possible to create biosensors whereby MVOCs interact with an electrode (similar in construction to the well-established glucose sensor where the surface consists of graphite particles, a polymer binder and other additives), changing surface properties allowing amount of compound to be instantly detected. To this end, unique profiles of MVOC for *Candida* growth on denture materials will be required for the development of a sensor capable of detecting colonization of dentures.

Methods

Gas chromatography of *C. albicans*

In order to investigate the MVOCs released by *C. albicans* biofilms, gas chromatography coupled with mass spectrometry and solid phase micro extraction (SPME GC-MS) was used. The column temperature was maintained at 70° C for 10 minutes, then increased to 270° C at a rate of 10° C per minute, and held for three minutes. The injector and detector temperatures were set to 270° C. The injector was split less with a flow rate set to 100 ml per minute, resulting in a run time of 33 minutes. This method was developed from a previous method used to identify volatile compounds produced by fungal isolates (Bingley et al., 2012). Blastospore and hyphal biofilms were examined at 24, 48 and 72 hours for the volatile compounds produced.

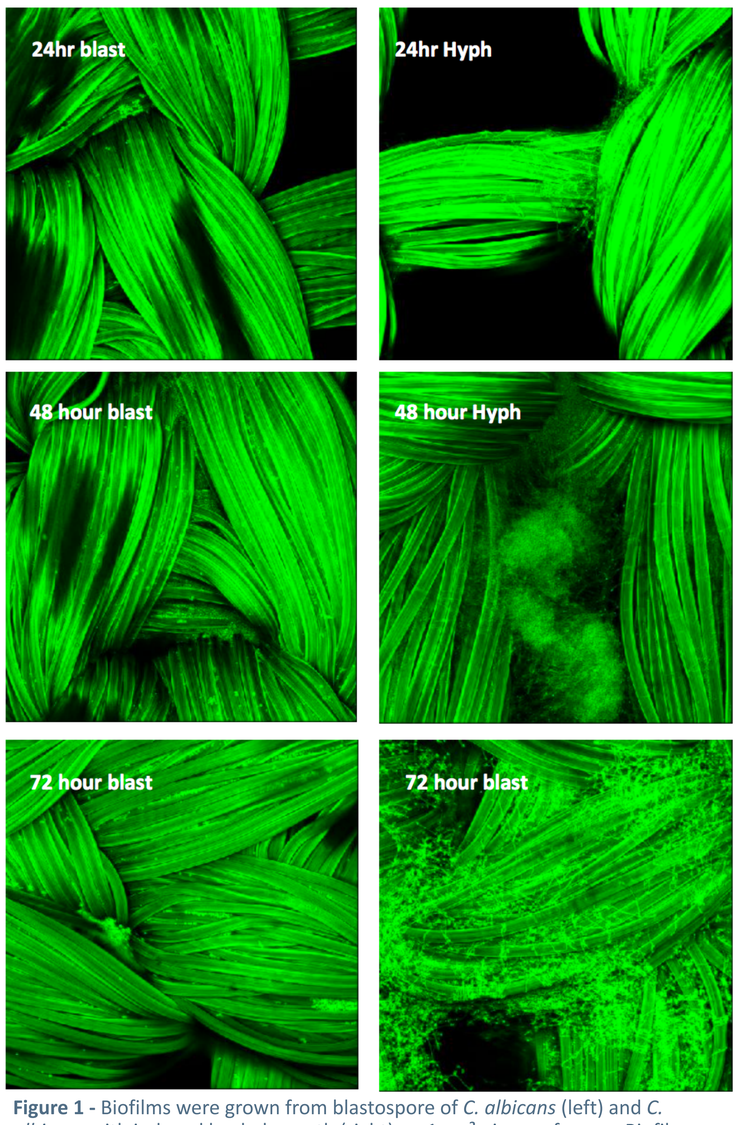

Figure 1 - Biofilms were grown from blastospore of *C. albicans* (left) and *C. albicans* with induced hyphal growth (right) on 1 cm² pieces of gauze. Biofilms were grown for 24 (top), 48 (middle) and 72 (bottom) hours.

Results and discussion

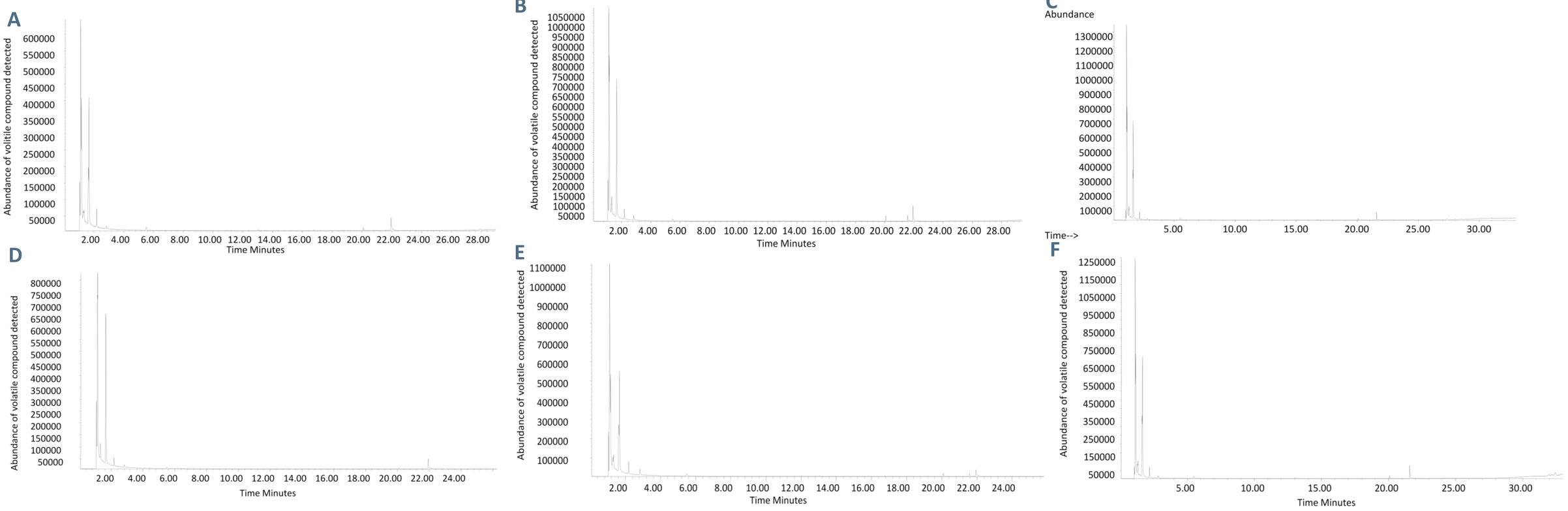

Figure 2 - Volatile compounds and potential quorum sensing molecules detected from A) 24 hour blastospore biofilms of *C. albicans* grown on gauze, B) 48 hour blastospore biofilms of *C. albicans* grown on gauze, C) 72 hour blastospore biofilms of *C. albicans* grown on gauze, D) 24 hour hyphal-induced biofilms of *C. albicans* grown on gauze, E) 24 hour hyphal-induced biofilms of *C. albicans,* F) 72 hour hyphal-induced biofilms of *C. albicans* grown on gauze

Blastospore and hyphal biofilms were examined at 24, 48 and 72 hours for the volatile compounds produced. At 24, 48 and 72 hours there was little difference between the volatile compounds detected from blastospore biofilms and those detected from hyphal biofilms. At 24 hours, both biofilm types produced ethanol and nerolidol (Figure 3). At 48 and 72 hours both biofilm types continued to yield peaks for ethanol and in addition to this peak representing farnesol type molecules were identified.

	24 hours		48 hours		72 hours	
	Blastospore	Hyphal	Blastospore	Hyphal	Blastospore	Hyphal
Carbon dioxide	4	4	48	4	4	
Ethanol	90	90	90	90	90	90
Acetic acid	38	49	49	49	72 hours	86
1 pentanol	83					
1 butanol	86	90	90	86	90	90
Nerolidol	87	91	90		78	
alpha farnesene			89			
Farnesol			40			
6,10 dodecatrien-3-ol				64		90
6-Octen-1-ol				40		
beta bisabolene				76		90
dodecadien 1-ol					54	

Figure 3 - Volatile compounds and potential quorum sensing molecules as analysed using gas chromatography and database referencing of chromatogram

Alem et al., 2006, reported the identification of tyrosol from early stage *C. albicans* biofilms and planktonic cultures. Tyrosol was not isolated from planktonic or biofilm cultures in this work which may be a result of a number of factors. Firstly, biofilms analysed in this work were only measured for detectable compounds at 24 and 48 hours of growth. Alem et al., detected tyrosol after 10 hours of growth in planktonic cultures and in 1-6 hour biofilms and noted that farnesol dominated in later stages of biofilm growth. In this study any tyrosol present and tyrosol production by cells at 24 and 48 hours may have been low in comparison to the amount of farnesol and therefore not detected.

Overall The SPME GC-MS method used in this study was successful in the identification of volatile and potential quorum sensing molecules produced by C. albicans. Further work will clarify a reproducible GCMS methodology to work towards the identification of a unique MVOC profile, when growing biofilms of denture (and other healthcare associated) materials.

References
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