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Review

In silico approaches in the identification of *Cryptococcus neoformans* chemoreceptors

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***Cryptococcus neoformans*, a pathogenic, yeast-like fungus, typically forms biofilms on medical devices that prevents the efficient penetration of antifungal drugs. As quorum sensing (QS)-related chemoreceptors play a crucial role in the pathogenesis of *Cryptococcus neoformans* particularly in biofilm formation, studying them would enable a clearer understanding on the host-pathogen interaction. However, information regarding *C. neoformans* QS-related chemoreceptors such as the encoding DNA sequences, protein structures and the binding ligands remain unknown till today. Here, we provide information to facilitate researchers to comprehend the basic views on gene homology and its various applications in the identification of novel chemoreceptors in microorganism especially the novel QS-related chemoreceptors for future use.**

Key words: *Cryptococcus neoformans*, homology-based transfer, quorum sensing, chemoreceptors.

INTRODUCTION

Cryptococcosis is the chief contributor (10 to 40%) of HIV-related deaths in the sub-Saharan Africa and Southeast Asia, a region where over 80% of the world's HIV-affected population lives (Liu et al., 2008). A global survey in 2006 estimated an approximate 957,900 cases of cryptococcal meningitis, leading to 624,700 deaths. Southeast Asia contributed approximately 120,000 of those cases and 66,000 fatalities (Park et al., 2009). As cryptococcosis is not a notifiable disease, the incidence count report would most probably be underestimated (Tay et al., 2009). Similar to *Candida albicans*, *C. neoformans* has the ability to form biofilm that would confer an increase in resistance towards antifungal drugs and host defense mechanisms (Robertson and Casadevall, 2009). Interestingly, the formation of biofilm can be triggered by environmental cues such as glucose, amino acids, pheromones, ammonium or small molecules produced at high cell densities (Bahn et al., 2007). Hence, the production and recognition of these extracellular signals, which are also known as quorum sensing, is vital in maintaining the population of cell growth and its functionality.

QUORUM SENSING

To date, the existence of quorum sensing has been extensively studied in prokaryotes such as in *Staphylococcus aureus* and *Pseudomonas aeruginosa*, but quorum sensing research in fungi is still relatively in its infancy. Over the recent years, the main focus for fungal quorum sensing research lies in *Candida spp* (Shchepin et al., 2003) and it has been reported that quorum-sensing molecules (QSMs) produced by *Candida spp* were able to innately control population growth according to cell density and may play important roles in *C. albicans* yeast-hypha morphology switching. In fact, QSM such as farnesol, a sesquiterpene produced by *C. albicans*, and *Pseudomonas aeruginosa* QSM, 3-oxo-C12 homoserine lactone (3OC12HSL), were found to repress fungal hypha formation (Hornby et al., 2001; Kulkarni and Nickerson, 1981). Nevertheless, the information on the chemoreceptors that recognize the molecules and the mechanism of internalization remained unknown (Rhome and Del Poeta, 2009). This warrants a more in-depth study particularly by using bioinformatics such as homology-based modeling in the hope to identify

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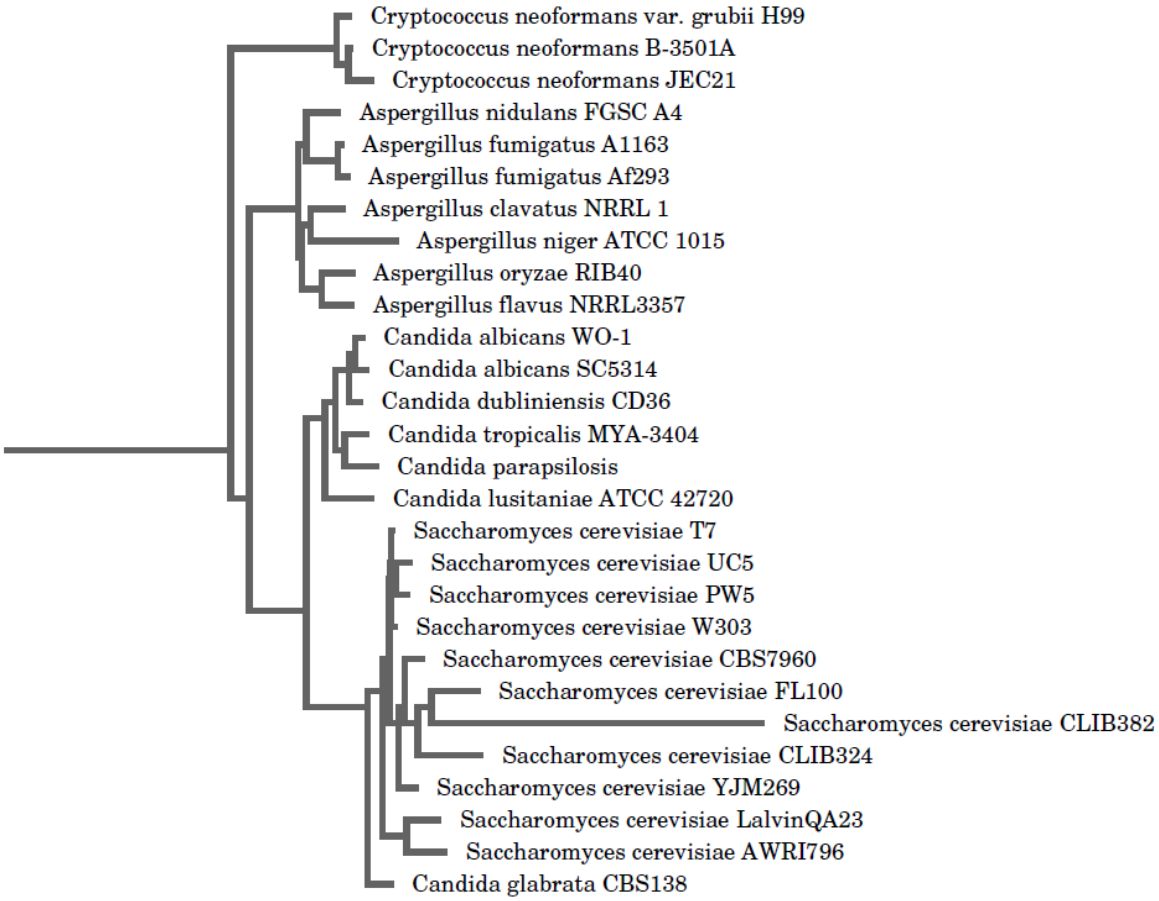

Figure 1. Phylogenetic comparisons of *C. neoformans*, *Aspergillus spp*, *Candida spp* and *S. cerevisae*. The tree shown represents degrees of similarities between these four species which emerged from a single major phylogenetic clade. The phylogenic tree was generated using the Clustal W2 software (Herrero, 2005).

novel chemoreceptors.

HOMOLOGY-BASED TRANSFER

In our model organism, cell-to-cell communication in *C. neoformans* occurs through the production and detection of signaling molecules (Ramage et al., 2009). Hence, homology-based transfer approaches which are able to assign unannotated genes or proteins with the functions of their homologues (Friedberg, 2006) can be utilized to predict the QS-related chemoreceptors genes in *C. neoformans.* This can be accomplished due to similarities in the sequences which the organism most likely evolved and would therefore have similar functions (Sleator and Walsh, 2010). Moreover, comparative gene prediction is a good strategy in fungi due to their relatively simple gene structures and the large clusters of related genomes (Galagan et al., 2005). For example, comparative genomics have established at least 65% similarity between the genomes of *Saccharomyces cerevisiae* and *C. neoformans* and the genetic differences between these two organisms are specifically due to certain phenotypic traits that are only present in *C. neoformans* such as the discrete synthesis of capsule and formation of melanin (Figure 1) (Herrero, 2005). Nevertheless, the high levels of gene conservation between *S. cerevisiae* and *C. neoformans* are remarkable especially for the essential genes such as glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Figure 2); although these two species diverged in the evolutionary tree for more than 1,000 million years ago (Herrero, 2005; Loftus et al., 2005). A notable improvement in homology-based modeling involves the combination of both sequence and structural-based homology transfer to determine novel chemoreceptor sequences. Glekas et al., (2010) have successfully utilized both sequence as well as structural-based homology transfer to discover a novel chemoreceptor sensing domain in *Bacillus subtilis* based on a better studied chemoreceptor in *Escherichia coli*. In addition, a successful method of chemoreceptor identification has been reported by Xiu et al., 2010 to identify and characterize olfactory chemoreceptors in *Spodoptera*

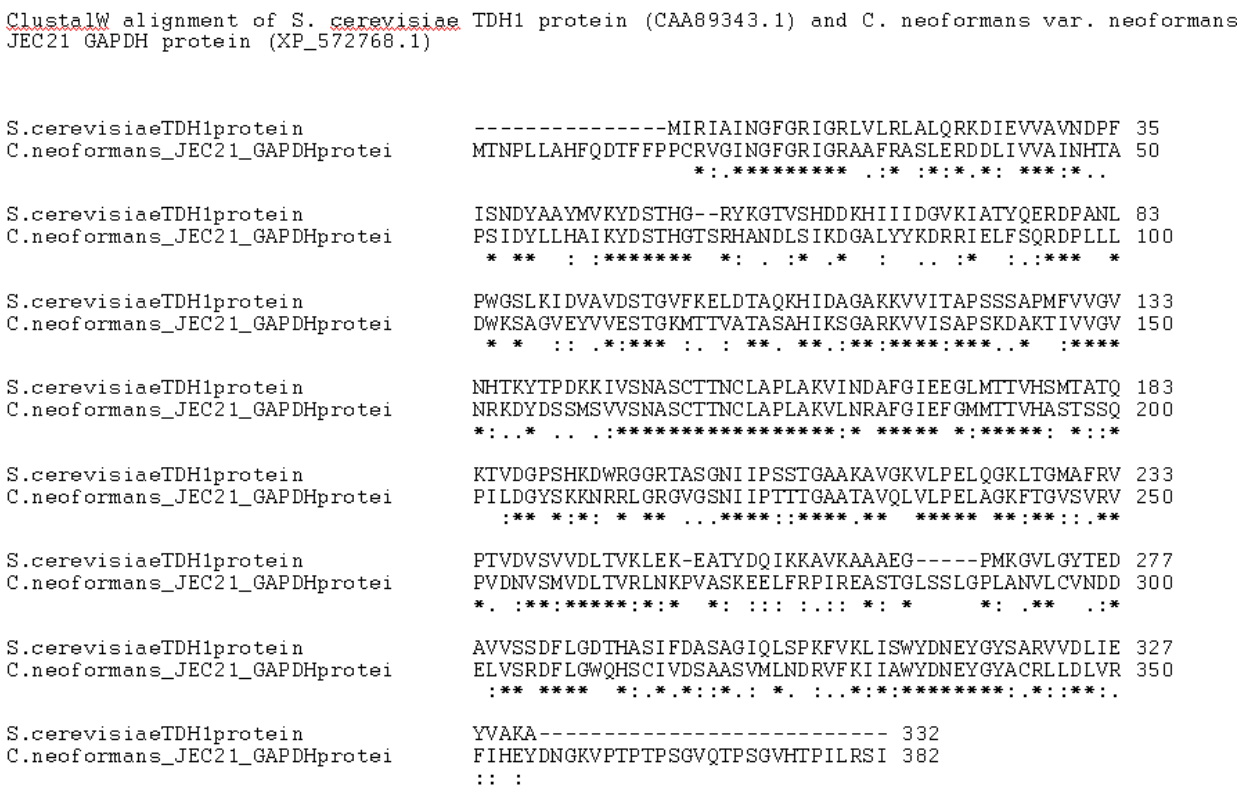

Figure 2. Multiple sequence alignment indicating amino acid sequence homology in glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein for *S. cerevisae* and *C. neoformans*. (Herrero, 2005; Loftus, 2005). *Indicates sequence conservation and numerous sequence similarity is conserved between these two species. The multiple sequence alignment was performed using the Clustal W software.

exigua and *Spodoptera litura* based on the knowledge of highly conserved chemoreceptors in *Drosophila melanogaster*. Their research group employed bioinformatics approaches to identify the desired sequences and web-based TMHMM software to determine the transmembrane regions of the chemoreceptor. In a separate study, web-based programs such as Sequencher v4.5 and TMPred can be used to predict and validate the olfactory chemoreceptor sequences obtained in *Aedes aegypti* (Bohbot et al., 2007). Previous discovery by Lee et al., 2007 unearthed a mutation of the global repressor Tup1 in a *C. neoformans* serotype D strain that led to the presence of a 11-mer peptide which ultimately enabled *C. neoformans* to grow on solid media only when the population density reaches 10^5 to 10^6 cells per plate.

CONCLUSION

Although we acknowledge that further research to discover these QS-related chemoreceptors is needed, we also found that the lack of information on the QS-related chemoreceptors in other fungi has hindered the progress of utilizing homology-based transfer to determine its homologues in *C. neoformans*. Nevertheless, we hope through the systematic understanding of QS-related chemoreceptors in *C. neoformans*, we would be able to utilize bioinformatics-based approaches coupled with molecular docking programs to identify suitable ligands or inhibitors that could potentially be used for antifungal drug design and to facilitate the identification of chemoreceptors in other medically important fungi. For instance, a research group utilized Autodock program to perform blind docking, using the sex pheromone bombykol and their analogs to predict the binding towards their cognate chemoreceptors in silkworm, *Bombyx mori* (He et al., 2010). Overall, we would highlight that this biotechnological concept is the way forward in identification of novel *C. neoformans* QS-related chemoreceptor genes in the effort to improve the prognostic outcome of cryptococcal infection and ultimately, the prevention of fatal cryptococcal meningoencephalitis in humans.

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