Karolinska Institutet

**Institutionen för Mikrobiologi, Tumör och Cell Biologi**

# Biofilm formation in invasive disease caused by fungus of the genus *Candida*

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Lennart Nilssonsalen, Nobel väg 15A

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av

**Srisuda Pannanusorn**

*Huvudhandledare:*
Professor Ute Römling
Karolinska Institutet
Institutionen för Mikrobiologi, Tumör-och Cellbiologi

*Bihandledare:*
Dr. Antonio Barragan
Smittskyddsinstitutet
Karolinska Institutet
Institutionen för Medicin, Huddinge
Centrum för infektionsmedicin

*Fakultetsopponent:*
Professor Geraldine Butler
University College Dublin,
Conway Institute of Biomolecular and Biomedical Research, Ireland

*Betygsnämnd:*
Professor Jan Sjölin
Uppsala Universitet
Institutionen för Medicinska Vetenskaper

Associate Professor Erja Chryssanthou
Karolinska Institutet
Institutionen för Mikrobiologi, Tumör-och Cellbiologi

Associate Professor Volkan Özenci
Karolinska Institutet
Institutionen för Laboratoriemedicin

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## ABSTRACT

*Candida* species are ranked as the fourth leading cause of nosocomial bloodstream infection. The association of the infection with several risk factors has been reported. The most predominant risk factor, however, is the presence of an intravenous catheter in patients, which provides an artificial surface for *Candida* spp. to form biofilms and subsequently initiate infection.

A total of 940 yeast isolates, mostly *Candida* spp., causing bloodstream infection in Sweden were collected between September 2005 – August 2006 and species identified through sequence polymorphisms in 40 nucleotides in the internal transcribed spacer region 2 (ITS2) of the ribosomal RNA gene obtained by pyrosequencing (paper I). Using this newly develop molecular approach, all isolates could be reliably identified down to the species level. In addition, intraspecies sequence variation in *Candida albicans* and *Candida glabrata* suggests subclassification of isolates. As *Candida* specification is associated with differences in susceptibility to antifungal drugs, accurate species identification aids treatment decisions. Pyrosequence analysis of the 40 nucleotides in the ITS2 region is fast and reliable, and can therefore contribute to the high quality management of patients with invasive fungal infections.

Biofilm formation of *Candida* spp. isolates was determined with a model mimicking the clinical conditions of the intravenous catheter surface through using a silicone elastomer, the host contribution through conditioning the surface with 10% human serum and the parenteral nutrition solution by a defined growth medium containing 10% glucose (paper II). Under these experimental conditions, biofilms of all *Candida* spp. with the exception of *Candida parapsilosis* were found to be composed of basal yeast cells. As a correlation between the amount of biofilm and the metabolic activity was observed, biofilm formation could be directly compared among *Candida* spp. isolates with isolates forming no, low and high biofilm. Biofilm formation was less prevalent in *C. albicans* isolates compared to non-*albicans Candida* spp. isolates. Thus, biofilm formation seems to be more significant for infections caused by non-*albicans Candida* species than by *C. albicans*. It is, therefore, possible that *C. albicans* uses mechanisms other than biofilm formation to cause bloodstream infection (paper II).

Among *Candida* spp., *C. parapsilosis* is the second/third most common cause of bloodstream infection. Biofilm formation of *C. parapsilosis* was highly variable among 33 epidemiologically independent isolates (paper II and paper V). A nosocomial outbreak involving *C. parapsilosis* has been reported from a hospital in Southern Sweden (paper III). The clonal origin of the *C. parapsilosis* isolates was confirmed. All outbreak isolates robustly showed high level of biofilm formation with a compelx structure on three surface materials, while expression of major virulence factors was low (paper IV). This finding indicated the significance of biofilm formation of *C. parapsilosis* as a determinative factor to cause the outbreak infection.

Within 33 clinical isolates of *C. parapsilosis* causing bloodstream infection, two different biofilm architectures could be identified in the 15 isolates forming high level of biofilm (paper V). Of these 15 isolates, 11 showed a complex structure biofilm consisting of macro-colonies with a spider-like appearance composed of aggregated yeast cells and pseudohyphae, while four isolates formed monolayers of pseudohyphae. Surprisingly, biofilm formation of isolates with high biofilm formation including an outbreak isolate were independent of the transcription factor Bcr1, a major biofilm regulator, although *BCR1* deletion affected colony switching and cell morphology in those isolates. As novel phenotypes, *BCR1* regulated secretion of aspartyl proteinases and susceptibility to antimicrobial peptides irrespectively of the biofilm formation phenotype in all tested isolates of *C. parapsilosis* (paper V).

In conclusion, work in this thesis characterised biofilm formation in an extensive number of *Candida* spp. isolates causing bloodstream infection. Detailed analysis of biofilm development in *C. parapsilosis* revealed that high biofilm formation in *C. parapsilosis* is independent of the major biofilm regulator Bcr1. This finding will have an impact on the development of biofilm prevention strategies in *C. parapsilosis*.


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