

THE EFFECT OF PHENOTYPIC
SWITCHING ON THE BIOLOGICAL
PROPERTIES OF *Candida krusei*

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ABSTRACT

Candida krusei has been identified as an emerging pathogen after *Candida albicans* and *Candida glabrata*. Until today, the ability to switch its phenotype in unfavourable environment has not been reported in *Candida krusei*. This study was carried out in order to evaluate the phenotypic switching ability of *Candida krusei* and to access how this ability affects the biological properties, adherence capacity and susceptibility towards chlorhexidine (CHX), amphotericin B, nystatin, *Piper betle* and *Nigella sativa* aqueous extracts. To induce the switched generations, *Candida krusei* was cultured on yeast extract potato dextrose (YEPD) agar containing 0.05% of phloxine B. Following a 5-day incubation, the colony forming units (CFU/mL) were examined and determined. This phenotypically switched colony was designated as the 1st cell generation. The cells from the 1st generation were subcultured following the same protocol to produce the 2nd, 3rd and 4th generation of switched cells.

The 1st and 2nd switched generations were observed to exhibit similar colony morphology comparative to the unswitched *Candida krusei*. The percentages of recovery population for the 1st and 2nd generations were reduced to 46.6% and 36.4%, respectively. The colonies from the 3rd and 4th switched generations were found to be highly myceliated with the former exhibiting lobate margin and the later with filamentous margin. Interestingly, the percentage of recovery for the 3rd generation showed a tremendous increased to 85.7% but was reduced to 70.8% in the 4th generation. SEM micrographs revealed the surface appearance of the unswitched *Candida krusei*, 1st and 2nd generations as smooth, with the 2nd generation having more extended pseudohyphae compared to the other generations. In contrast, the surface of the 3rd and the 4th generations were with rough surfaces. The 4th generation also exhibited pimped or punctate morphology with short pseudohyphae. The unswitched *Candida krusei* and the 3rd switched generations were also observed to have deposits of

extracellular matrix on its surfaces. The adherence capacity of *Candida krusei* also showed variations in all cell generations. The 2nd switched generation showed the highest adherence with total population of $(154.0 \pm 60.2) \times 10^2$ CFU/mL to the saliva-coated glass beads while the unswitched *Candida krusei* showed the least adherence at $(5.65 \pm 0.5) \times 10^2$ CFU/mL.

Based on the disc diffusion test, the degree of susceptibility towards CHX, amphotericin B, nystatin and *Piper betle* were found to differ in all generations of *Candida krusei*. The unswitched *Candida krusei* was found to be the most susceptible towards CHX and the 2nd generation was the least susceptible. The 3rd unswitched *Candida krusei* was found to be the most susceptible towards amphotericin B and the unswitched generation was the least susceptible. The 4th generation was determined as the most susceptible towards nystatin in contrast to the 2nd generation which showed the least. In the susceptibility study towards *Piper betle* results indicated that the 1st generation was the most susceptible while the 4th generation was the least. The MIC and MFC of *Candida krusei* for the unswitched and all switched generations towards CHX, amphotericin B, nystatin and *Piper betle* were determined at 0.4 µg/µL, 50 µg/mL, 10 unit/mL and 12.5 mg/mL respectively. From the growth curve study, the unswitched and all switched generations of *Candida krusei* showed varying degree of responses towards CHX, amphotericin B and *Piper betle* treated environment.

These results suggested that *Candida krusei* is able to switching ability is a virulence factor of *Candida krusei* which affects the biological properties, adherence ability and susceptibility towards CHX, amphotericin B, nystatin and *Piper betle*. Thus, it leads to the pathogenic property in the oral cavity.

ABSTRAK

Candida krusei semakin dikenalpasti sebagai patogen selepas *Candida albicans* dan *Candida glabrata*. Sehingga kini, keupayaannya untuk mengubah fenotip masih belum diketahui. Justeru, kajian ini telah dijalankan bagi menilai keupayaan *Candida krusei* untuk mengubah fenotip seterusnya mengenalpasti kesan perubahan tersebut terhadap sifat biologi, perlekatan dan kerentanan terhadap chlorhexidine (CHX), amphotericin B, nystatin, ekstrak akues *Piper betle* dan *Nigella sativa*. Untuk menggalakkan perubahan fenotip, *Candida krusei* telah dikulturkan pada agar Yis Ekstrak Pepton Dektrosa (YEED) yang mengandungi 0.05% phloxine B. Selepas eraman selama 5 hari, unit pembentukan koloni (CFU/mL) dinilai. Koloni yang mengandungi perubahan fenotip ini dianggap sebagai generasi pertama. Koloni ini telah dikulturkan semula mengikut prosedur yang telah ditetapkan bagi pembentukan generasi kedua, ketiga dan keempat.

Candida krusei generasi pertama dan kedua dilihat mempunyai morfologi yang sama seperti *Candida krusei* yang asal. Peratus koloni yang tumbuh bagi generasi pertama dan kedua dilihat menurun kepada 46.6% dan 36.4% masing-masing. Generasi ketiga dan keempat pula didapati membentuk banyak miselia. Margin berbentuk lobat telah dilihat pada generasi ketiga manakala bentuk filamen pula dilihat pada generasi keempat. Peratus koloni yang tumbuh pada generasi ketiga menunjukkan peningkatan yang ketara sebanyak 85.7% namun penurunan mendadak telah dilihat pada generasi keempat sebanyak 70.8%. Mikrograf SEM telah menunjukkan permukaan sel bagi *Candida krusei* yang belum berubah, generasi pertama, dan kedua adalah licin dengan generasi kedua menunjukkan sifat hifa palsu yang lebih panjang berbanding generasi lain. Sebaliknya, generasi ketiga dan keempat dilihat mempunyai struktur permukaan yang kasar. Generasi keempat turut dilihat membentuk permukaan yang seolah-olah berjerawat dan berbintik-bintik. Manakala generasi ketiga pula dilihat membentuk

matriks luar sel. Keupayaan *Candida krusei* untuk melekat didapati berbeza pada setiap generasi. Generasi kedua menunjukkan jumlah perlekatan tertinggi sebanyak $(154.0 \pm 60.2) \times 10^2$ CFU/mL terhadap butir kaca bersalut air liur manakala *Candida krusei* asal adalah yang terendah dengan $(5.65 \pm 0.5) \times 10^2$ CFU/mL.

Mengikut kajian penyerapan cakera, darjah kerentanan terhadap CHX, amphotericin B, nystatin dan *Piper betle* dilihat berbeza bagi semua generasi *Candida krusei*. *Candida krusei* asal didapati menunjukkan kerentanan yang tertinggi manakala generasi kedua pula adalah yang terendah. Generasi ketiga dilihat mempunyai kerentanan yang tertinggi terhadap amphotericin B manakala *Candida krusei* asal dilihat mempunyai darjah kerentanan yang terendah. Kajian kerentanan terhadap nystatin menunjukkan generasi keempat mempunyai kerentanan tertinggi manakala generasi kedua adalah sebaliknya. Kajian terhadap ekstrak *Piper betle* menunjukkan generasi pertama mempunyai kerentanan tertinggi manakala generasi keempat adalah yang terendah. MIC dan MFC bagi *Candida krusei* asal dan setiap generasi terhadap CHX, amphotericin B, nystatin dan *Piper betle* adalah didapati pada 0.4 µg/µL, 50 µg/mL, 10 unit/mL, 12.5 mg/mL masing-masing. Kajian terhadap lengkok kehidupan *Candida krusei* dan setiap generasi terhadap CHX, amphotericin B dan *Piper betle* menunjukkan kepelbagaian tindakbalas terhadap agen anti-mikrobial.

Hasil kajian ini telah menunjukkan bahawa keupayaan *Candida krusei* untuk mengubah fenotip telah mempengaruhi sifat biologi, perlekatan dan kerentanan terhadap chlorhexidine (CHX), amphotericin B, nystatin, ekstrak akues *Piper betle* dan *Nigella sativa*. Justeru itu, keupayaan ini dilihat amat penting dalam memastikan kejayaannya sebagai patogen di dalam kaviti mulut.

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ABBREVIATIONS

%	:	percentage
°C	:	degree centigrade
µg	:	microgram
g	:	gram
µm	:	micrometer
L	:	liter
ml	:	milliliter
v/v	:	volume/volume
w/v	:	weight/volume
M	:	Molar
<i>ed.</i>	:	edition
<i>et al.</i>	:	et alia (and others)
no.	:	number
sp.	:	species
CHX	:	chlorhexidine
SEM	:	scanning electron microscope
GCF	:	gingival crevicular fluid
PBS	:	phosphate-buffered solution
CFU	:	colony forming unit
GABA	:	γ-aminobutyric acid