

**EFFECTS OF CAMPTOTHECIN ON *PICHIA PASTORIS* STRAIN SMD1168H EXPRESSING  
DNA TOPOISOMERASE I  
ON AGAR PLATES**

**SAJETRA DEVI VADIVELOO**

**UNIVERSITI SAINS MALAYSIA**

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**PUSAT PENGAJIAN TEKNOLOGI INDUSTRI  
UNIVERSITI SAINS MALAYSIA**

**BORANG PENYERAHAN DISERTASI MUTAKHIR  
SATU (1) NASKAH**

Nama Penyelia: Prof. Dr Liong Min Tze

Bahagian: Teknologi Bioproses

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Encik/Puan/Cik Sajetra Devi A/P Vadiveloo

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Sekian, terima kasih.

  
**PROF. DR LIONG MIN TZE**  
Ph.D. (Melb) Australia  
School of Industrial Technology  
Universiti Sains Malaysia. (USM)  
11800 Penang, Malaysia

20/07/2020

Tarikh



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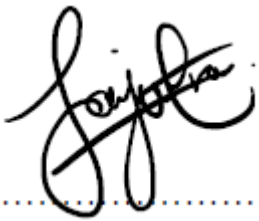
By

**SAJETRA DEVI VADIVELOO**

A dissertation submitted in the partial fulfillment of the requirements for the degree  
of Bachelor of Technology (B.Tech) in the field of Bioprocess Technology  
School of Industrial Technology  
Universiti Sains Malaysia  
July 2020

## **DECLARATION BY AUTHOR**

This dissertation is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.

A handwritten signature in black ink, appearing to read 'Sajetra', written over a horizontal dotted line.

SAJETRA DEVI A/P VADIVELOO

JULY 2020

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Sajetra Devi A/P Vadiveloo

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## TABLE OF CONTENTS

	Page
DECLARATION BY THE AUTHOR	ii
ACKNOWLEDGEMENTS	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	vii
LIST OF FIGURES	ix
LIST OF SYMBOLS	xii
LIST OF ABBREVIATIONS	xii
ABSTRAK	xv
ABSTRACT	xvi
CHAPTER 1 INTRODUCTION	
1.1 Research background	1
1.2 Rationale	3
1.3 Research Objectives	4
CHAPTER 2 LITERATURE REVIEW	
2.1 Overview of <i>Pichia pastoris</i>	5
2.1.1 <i>Pichia pastoris</i> expression system	7
2.1.2 Methanol metabolism	9
2.1.3 Preservative methods for <i>Pichia pastoris</i> transformants	12
2.1.4 Culturing <i>Pichia pastoris</i> in liquid and gel media	13
2.2 Overview of DNA topoisomerases	14
2.2.1 Classification of DNA topoisomerases	15

2.2.2 Applications of DNA topoisomerase I	16
2.3 Effect of camptothecin on DNA topoisomerase I	17
<b>CHAPTER 3 MATERIALS AND METHODS</b>	
3.1 Preparation of stock solutions	19
3.1.1 Dextrose (10x)	19
3.1.2 Dipotassium phosphate (1.0 M)	19
3.1.3 Monopotassium phosphate (1.0 M)	19
3.1.4 Potassium phosphate buffer (1.0 M, pH 6.0)	19
3.1.5 Yeast nitrogen base (10x)	20
3.1.6 Biotin (500x)	20
3.1.7 Glycerol (10x)	20
3.2 Preparation of media for <i>Pichia pastoris</i>	20
3.2.1 Yeast peptone dextrose (YPD) broth	20
3.2.2 Buffered glycerol complex medium (BMGY)	20
3.3 Preparation of reagents for multi-copy insert screening	21
3.3.1 Freezing media for glycerol stock (50%)	21
3.3.2 Camptothecin stock (10 mM)	21
3.3.3 YPD plate prepared using microbiological agar	21
3.3.4 YPD plate prepared using yeast extract agar	22
3.4 Growing multi-copy number insert of <i>P. pastoris</i> transformants	22
3.5 Spotting of <i>P. pastoris</i> transformants in different concentrations of camptothecin plates	24
3.6 Statistical Analysis	25
<b>CHAPTER 4 RESULTS AND DISCUSSION</b>	
4.1 Growth of multi-copy number insert of <i>P. pastoris</i> transformants	26

4.2 Growth of the recombinant clones on yeast extract agar and microbiological plate	27
4.2.1 Control (without camptothecin)	27
4.2.2 Camptothecin (25 $\mu$ M)	37
4.2.3 Camptothecin (50 $\mu$ M)	46
4.2.4 Camptothecin (75 $\mu$ M)	55
4.2.5 Camptothecin (100 $\mu$ M)	64
<b>CHAPTER 5 CONCLUSION</b>	
5.1 Conclusion	75
5.2 Recommendations for Future Research	75
<b>REFERENCES</b>	76
<b>APPENDIX</b>	80



## LIST OF TABLES

Table Caption	Page
2.1 Wide range of <i>Pichia pastoris</i> strains used for heterologous protein expression (Higgins, 1995).	8
2.2 Subfamilies of DNA topoisomerases (Wang, 2002).	15
4.1 Absorbance reading of the recombinant clones of <i>Pichia pastoris</i> transformants (SMD1168H pPICZ $\alpha$ -A-hTopoI) after overnight incubation.	26
4.2 Average intensity of the recombinant clone A of multi-copy number insert of <i>Pichia pastoris</i> (SMD1168H pPICZ $\alpha$ -A-hTopoI). The clones were grown on yeast extract agar plate and microbiological agar plate without camptothecin (control) on first, second and third days of incubation, respectively.	29
4.3 Average intensity of the recombinant clone B of multi-copy number insert of <i>Pichia pastoris</i> (SMD1168H pPICZ $\alpha$ -A-hTopoI). The clones were grown on yeast extract agar plate and microbiological agar plate without camptothecin (control) on first, second and third days of incubation, respectively.	32
4.4 Average intensity of the recombinant clone A of multi-copy number insert of <i>Pichia pastoris</i> (SMD1168H pPICZ $\alpha$ -A-hTopoI). The clones were grown on 25 $\mu$ M camptothecin of yeast extract agar plate and microbiological agar plate on first, second and third days of incubation, respectively.	38
4.5 Average intensity of the recombinant clone B of multi-copy number insert of <i>Pichia pastoris</i> (SMD1168H pPICZ $\alpha$ -A-hTopoI). The clones were grown on 25 $\mu$ M camptothecin of yeast extract agar plate and microbiological agar plate on first, second and third days of incubation, respectively.	41
4.6 Average intensity of the recombinant clone A of multi-copy number insert of <i>Pichia pastoris</i> (SMD1168H pPICZ $\alpha$ -A-hTopoI). The clones were grown on 50 $\mu$ M camptothecin of yeast extract agar plate and microbiological agar plate on first, second and third days of incubation, respectively.	47
4.7 Average intensity of the recombinant clone B of multi-copy number insert of <i>Pichia pastoris</i> (SMD1168H pPICZ $\alpha$ -A-hTopoI). The clones were grown on 50 $\mu$ M camptothecin of yeast extract agar plates and microbiological agar plate on first, second and third days of incubation, respectively.	50

## Lists of Tables (Continued).

<b>Table Caption</b>	<b>Page</b>
4.8 Average intensity of the recombinant clone A of multi-copy number insert of <i>Pichia pastoris</i> (SMD1168H pPICZ $\alpha$ -A-hTopoI). The clones were grown on 75 $\mu$ M camptothecin of yeast extract agar plate and microbiological agar plate on first, second and third days of incubation, respectively.	56
4.9 Average intensity of the recombinant clone B of multi-copy number insert of <i>Pichia pastoris</i> (SMD1168H pPICZ $\alpha$ -A-hTopoI). The clones were grown on 75 $\mu$ M camptothecin of yeast extract agar plate and microbiological agar plate on first, second and third days of incubation, respectively.	59
4.10 Average intensity of the recombinant clone A of multi-copy number insert of <i>Pichia pastoris</i> (SMD1168H pPICZ $\alpha$ -A-hTopoI). The clones were grown on 100 $\mu$ M camptothecin of yeast extract agar plate and microbiological agar plate on first, second and third days of incubation, respectively.	65
4.11 Average intensity of the recombinant clone B of multi-copy number insert of <i>Pichia pastoris</i> (SMD1168H pPICZ $\alpha$ -A-hTopoI). The clones were grown on 100 $\mu$ M camptothecin of yeast extract agar plate and microbiological agar plate on first, second and third days of incubation, respectively.	68

## LIST OF FIGURES

Figure Caption	Page
2.1 The methanol pathway in <i>Pichia pastoris</i> . 1: alcohol oxidase; 2: catalase; 3: formaldehyde dehydrogenase; 4: formate dehydrogenase, 5 di-hydroxyacetone synthase; 6: dihydroxyacetone kinase; 7: fructose 1,6-biphosphate aldolase; 8: fructose 1,6-bisphosphatase (Cereghino & Cregg, 2000).	10
3.1 The diagram of a microplate, where serial dilution was carried out. Indicator X displays increased series of dilution with a dilution factor of 0.05 (1 <sup>o</sup> ), 2.5×10 <sup>-3</sup> (2 <sup>o</sup> ), 1.25×10 <sup>-4</sup> (3 <sup>o</sup> ), 6.25×10 <sup>-6</sup> (4 <sup>o</sup> ), 3.13×10 <sup>-7</sup> (5 <sup>o</sup> ), 1.56×10 <sup>-8</sup> (6 <sup>o</sup> ) and 7.81×10 <sup>-10</sup> (7 <sup>o</sup> ), respectively, whereas indicator Y displays the labelling of clones for <i>P. pastoris</i> transformants. Rows A1, A2 and A3 are the triplications of clone A, whereas rows B1, B2 and B3 are the triplications of clone B. Zone Z displays the series of cultures that were used to spot on the agar plates in the following sections.	23
3.2 The diagrams of petri dishes, where the clones of <i>Pichia pastoris</i> transformants were spotted in triplicates following the dilution factor of 3.13×10 <sup>-7</sup> , 1.56×10 <sup>-8</sup> , and 7.81×10 <sup>-10</sup> : (a) microbiological agar and (b) yeast extract agar plates. Rows A1, A2 and A3 are the triplications of clone A, whereas rows B1, B2 and B3 are the triplications of clone B.	24
4.1 Growth of the recombinant clones of multi-copy number insert of <i>Pichia pastoris</i> transformants. X: BMGY inoculated with recombinant clone A; Y: BMGY inoculated with recombinant clone B; and Z: non-inoculated BMGY (control).	27
4.2 The average intensity (growth) of the recombinant clone A of multi-copy number insert of <i>Pichia pastoris</i> transformants grown on yeast extract and microbiological agar plates without camptothecin (control) at first, second and third days of incubation.	30
4.3 The average intensity (growth) of the recombinant clone B of multi-copy number insert of <i>Pichia pastoris</i> transformants on yeast extract and microbiological agar without camptothecin (control) plates at first, second and third days of incubation.	33

## Lists of Figures (Continued).

Figure Caption	Page
4.4 Yeast extract agar plates without camptothecin (control). The growth of two recombinant clones (A and B) of <i>Pichia pastoris</i> transformants on the plates for (a) first day, (b) second day, and (c) third day. X: clones spotted at a dilution factor of $3.13 \times 10^{-7}$ ; Y: clones spotted at a dilution factor of $1.56 \times 10^{-8}$ ; Z: clones spotted at a dilution factor of $7.81 \times 10^{-10}$ . Each recombinant was spotted in triplicates prior dilution.	35
4.5 Microbiological agar plates without camptothecin (control). The growth of two recombinant clones (A and B) of <i>Pichia pastoris</i> transformants on the plates for (a) first day, (b) second day, and (c) third day. X: clones spotted at a dilution factor of $3.13 \times 10^{-7}$ ; Y: clones spotted at a dilution factor of $1.56 \times 10^{-8}$ ; Z: clones spotted at a dilution factor of $7.81 \times 10^{-10}$ . Each recombinant was spotted in triplicates prior dilution.	36
4.6 The average intensity (growth) of the recombinant clone A of multi-copy number insert of <i>Pichia pastoris</i> transformants on 25 $\mu$ M camptothecin of yeast extract and microbiological agar plates at first, second and third days of incubation.	39
4.7 The average intensity (growth) of the recombinant clone B of multi-copy number insert of <i>Pichia pastoris</i> transformants on 25 $\mu$ M camptothecin of yeast extract and microbiological agar plates at first, second and third days of incubation.	42
4.8 Yeast extract agar plates that contained 25 $\mu$ M of camptothecin. The growth of two recombinant clones (A and B) of <i>Pichia pastoris</i> transformants on the plates for (a) first day, (b) second day, and (c) third day. X: clones spotted at a dilution factor of $3.13 \times 10^{-7}$ ; Y: clones spotted at a dilution factor of $1.56 \times 10^{-8}$ ; Z: clones spotted at a dilution factor of $7.81 \times 10^{-10}$ . Each recombinant was spotted in triplicates prior dilution.	44
4.9 Microbiological agar plates that contained 25 $\mu$ M of camptothecin. The growth of two recombinant clones (A and B) of <i>Pichia pastoris</i> transformants on the plates for (a) first day, (b) second day, and (c) third day. X: clones spotted at a dilution factor of $3.13 \times 10^{-7}$ ; Y: clones spotted at a dilution factor of $1.56 \times 10^{-8}$ ; Z: clones spotted at a dilution factor of $7.81 \times 10^{-10}$ . Each recombinant was spotted in triplicates prior dilution.	45

## Lists of Figures (Continued).

Figure Caption	Page
4.10 The average intensity (growth) of the recombinant clone A of multi-copy number insert of <i>Pichia pastoris</i> transformants on 50 $\mu$ M camptothecin of yeast extract and microbiological agar plates at first, second and third days of incubation.	48
4.11 The average intensity (growth) of the recombinant clone B of multi-copy number insert of <i>Pichia pastoris</i> transformants on 50 $\mu$ M camptothecin of yeast extract and microbiological agar plates at first, second and third days of incubation.	51
4.12 Yeast extract agar plates that contained 50 $\mu$ M of camptothecin. The growth of two recombinant clones (A and B) of <i>Pichia pastoris</i> transformants on the plates for (a) first day, (b) second day, and (c) third day. X: clones spotted at a dilution factor of $3.13 \times 10^{-7}$ ; Y: clones spotted at a dilution factor of $1.56 \times 10^{-8}$ ; Z: clones spotted at a dilution factor of $7.81 \times 10^{-10}$ . Each recombinant was spotted in triplicates prior dilution.	53
4.13 Microbiological agar plates that contained 50 $\mu$ M of camptothecin. The growth of two recombinant clones (A and B) of <i>Pichia pastoris</i> transformants on the plates for (a) first day, (b) second day, and (c) third day. X: clones spotted at a dilution factor of $3.13 \times 10^{-7}$ ; Y: clones spotted at a dilution factor of $1.56 \times 10^{-8}$ ; Z: clones spotted at a dilution factor of $7.81 \times 10^{-10}$ . Each recombinant was spotted in triplicates prior dilution.	54
4.14 The average intensity (growth) of the recombinant clone A of multi-copy number insert of <i>Pichia pastoris</i> transformants on 75 $\mu$ M camptothecin of yeast extract and microbiological agar plates at first, second and third days of incubation.	57
4.15 The average intensity (growth) of the recombinant clone B of multi-copy number insert of <i>Pichia pastoris</i> transformants on 75 $\mu$ M camptothecin of yeast extract and microbiological agar plates at first, second and third days of incubation.	60
4.16 Yeast extract agar plates that contained 75 $\mu$ M of camptothecin. The growth of two recombinant clones (A and B) of <i>Pichia pastoris</i> transformants on the plates for (a) first day, (b) second day, and (c) third day. X: clones spotted at a dilution factor of $3.13 \times 10^{-7}$ ; Y: clones spotted at a dilution factor of $1.56 \times 10^{-8}$ ; Z: clones spotted at a dilution factor of $7.81 \times 10^{-10}$ . Each recombinant was spotted in triplicates prior dilution.	62

## Lists of Figures (Continued).

Figure Caption	Page
4.17 Microbiological agar plates that contained 75 $\mu\text{M}$ of camptothecin. The growth of two recombinant clones (A and B) of <i>Pichia pastoris</i> transformants on the plates for (a) first day, (b) second day, and (c) third day. X: clones spotted at a dilution factor of $3.13 \times 10^{-7}$ ; Y: clones spotted at a dilution factor of $1.56 \times 10^{-8}$ ; Z: clones spotted at a dilution factor of $7.81 \times 10^{-10}$ . Each recombinant was spotted in triplicates prior dilution.	63
4.18 The average intensity (growth) of the recombinant clone A of multi-copy number insert of <i>Pichia pastoris</i> transformants on 100 $\mu\text{M}$ camptothecin of yeast extract and microbiological agar plates at first, second and third days of incubation.	66
4.19 The average intensity (growth) of the recombinant clone B of multi-copy number insert of <i>Pichia pastoris</i> transformants on 75 $\mu\text{M}$ camptothecin of yeast extract and microbiological agar plates at first, second and third days of incubation.	69
4.20 Yeast extract agar plates that contained 100 $\mu\text{M}$ of camptothecin. The growth of two recombinant clones (A and B) of <i>Pichia pastoris</i> transformants on the plates for (a) first day, (b) second day, and (c) third day. X: clones spotted at a dilution factor of $3.13 \times 10^{-7}$ ; Y: clones spotted at a dilution factor of $1.56 \times 10^{-8}$ ; Z: clones spotted at a dilution factor of $7.81 \times 10^{-10}$ . Each recombinant was spotted in triplicates prior dilution.	71
4.21 Microbiological agar plates that contained 100 $\mu\text{M}$ of camptothecin. The growth of two recombinant clones (A and B) of <i>Pichia pastoris</i> transformants on the plates for (a) first day, (b) second day, and (c) third day. X: clones spotted at a dilution factor of $3.13 \times 10^{-7}$ ; Y: clones spotted at a dilution factor of $1.56 \times 10^{-8}$ ; Z: clones spotted at a dilution factor of $7.81 \times 10^{-10}$ . Each recombinant was spotted in triplicates prior dilution.	72

## LIST OF SYMBOLS AND ABBREVIATIONS

<b>Symbol</b>	<b>Caption</b>
+	Plus
-	Minus
%	Percentage
$\alpha$	Alpha
$\pm$	Plus-minus
<	Less than
$^{\circ}\text{C}$	Degree Celsius
<b>Abbreviation</b>	<b>Caption</b>
AOX1	Alcohol oxidase 1
AOX2	Alcohol oxidase 2
BMGY	Buffered glycerol complex medium
CPT	Camptothecin
CPT-11	Irinotecan
dH <sub>2</sub> O	Deionized water
DF	Dilution factor
DMSO	Dimethyl sulfoxide
DHA	Dihydroxyacetone synthase
DNA	Deoxyribonucleic acid
g	Gram
G3P	Glyceraldehyde 3-phosphate

## **Lists of Abbreviations (Continued).**

<b>Abbreviation</b>	<b>Caption</b>
hTopoI	Human Type I DNA Topoisomerase
K <sub>2</sub> HPO <sub>4</sub>	Dipotassium phosphate
KH <sub>2</sub> PO <sub>4</sub>	Monopotassium phosphate
LDH	Lactate dehydrogenase
mg	Milligram
ml	Milliliter
Mut+	Methanol utilization plus
OD	Optical density
TopoI	DNA topoisomerase I
TopoIcc	Topoisomerase I cleavage complex
TopoII	DNA topoisomerase II
rpm	Revolutions per minute
RNA	Ribonucleic acid
SCP	Single-cell protein
V <sub>i</sub>	Volume initial
V <sub>f</sub>	Volume final
v/v	Volume per volume
YNB	Yeast nitrogen base
YPD	Yeast peptone dextrose
μM	Micromolar
μl	Microliter



# KESAN CAMPTOTHECIN TERHADAP *PICHIA PASTORIS* STRAIN SMD1168H MENGESPRESSIKAN DNA TOPOISOMERASE I PADA AGAR

## ABSTRAK

DNA topoisomerase I (TopoI) merupakan sejenis enzim yang bertanggungjawab untuk mengurangkan tekanan topologi dengan memperkenalkan putusan sementara dalam heliks DNA. TopoI juga terlibat dalam percambahan sel; justeru, ekspresi berlebihan dalam sel daripada enzim ini sering meniru sel barah. Enzim ini juga memainkan peranan penting dalam kajian biologi molekul untuk membangunkan pelbagai jenis agen antineoplastik, seperti camptothecin. Camptothecin mempunyai sifat anti-perencatan yang kuat terhadap aktiviti pemangkin TopoI dengan mencegah proses re-ligasi DNA dan akan mengakibatkan kematian sel. Sistem ekspresi *Pichia pastoris* sangat terkenal berbanding sistem ekspresi yang lain seperti *Escherichia coli*, *Saccharomyces cerevisiae* dan baculovirus, kerana kemampuannya untuk menghasilkan enzim manusia (TopoI) Pada masa ini, pencarian sebatian yang lebih berkesan tanpa mewujudkan sebarang kesan sampingan yang dialami semasa rawatan kemoterapi adalah amat digalakkan. Maka, penyelidikan ini bertujuan untuk menyiasat sifat penghambatan camptothecin terhadap pertumbuhan klon rekombinan sisipan nombor pelbagai salinan transformer *P. pastoris* yang mengekspresikan DNA topoisomerase manusia (SMD1168H-pPICZ $\alpha$ A-hTopoI) yang diinokulasikan pada agar ekstrak yis dan agar mikrobiologi. Kedua-dua jenis agar tersebut telah disediakan dengan campuran camptothecin yang mempunyai konsentrasi yang berbeza, (25  $\mu$ M, 50  $\mu$ M, 75  $\mu$ M dan 100  $\mu$ M) dan dibiarkan selama 3 hari. Kesimpulannya, aktiviti perencatan tertinggi telah didapati pada kedua-dua klon rekombinan yang tumbuh dalam agar mikrobiologi yang mengandungi 100  $\mu$ M camptothecin.

**EFFECTS OF CAMPTOTHECIN ON *PICHIA PASTORIS* STRAIN  
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ON AGAR PLATES**

**ABSTRACT**

DNA topoisomerase I (TopoI) is a ubiquitous enzyme, that is responsible for releasing topological stress by introducing a temporary nick in one strand of the DNA helix and later, resealing the single-stranded DNA. TopoI is involved in cell proliferation; therefore, overexpression of this enzyme in a cell often mimics cancer cells. Hence, this enzyme plays a major role in molecular biology studies for developing various types of antineoplastic agents, such as camptothecin. Camptothecin exhibits strong anti-inhibitory properties towards the catalytic activity of TopoI by preventing the re-ligation of the nicked DNA, resulting in shear stress and eventually, cell death. *Pichia pastoris* expression system is well-known for its ability to produce human-like endogenous TopoI compared to other expression systems, e.g. *Escherichia coli*, *Saccharomyces cerevisiae* and baculovirus. Currently, searching for more effective compounds to reduce the toxicity of cancer treatments, while still producing similar effects as current chemotherapy regimens is required. As such, this research aims to investigate the inhibitory properties of camptothecin on the growth of recombinant clones of multi-copy number insert of *P. pastoris* transformants expressing human DNA topoisomerase I (SMD1168H-pPICZ $\alpha$ A-hTopoI) grown on yeast extract agar and microbiological agar plates, respectively. The agar plates contained different concentrations of camptothecin (25  $\mu$ M, 50  $\mu$ M, 75  $\mu$ M and 100  $\mu$ M) and were left to incubate for 3 days. In conclusion, the highest inhibitory activity of camptothecin was observed when both the recombinant clones were grown in microbiological agar plate that contained 100  $\mu$ M of camptothecin.