

Contents

	Page
Abstract (Thai)	(3)
Abstract (English)	(5)
Acknowledgement	(7)
Contents	(9)
List of Tables	(13)
List of Figures	(15)
List of Abbreviations	(17)
Chapter	
1. Introduction	1
Literature review	3
1. <i>Schizophyllum commune</i>	3
1.1 General characteristic	3
1.2 The utilization of <i>S. commune</i>	4
1.2.1 As a food source	4
1.2.2 As a source of useful metabolites	4
2. Fibrinolytic enzyme	6
2.1 Conversion of fibrinogen to fibrin and fibrinolysis	6
2.2 Sources of fibrinolytic enzymes	9
2.3 Factors affecting on fibrinolytic enzyme production	12
2.3.1 Cultural conditions	12
2.3.2 Nutrient compositions	12
2.3.3 Temperature	13
2.3.4 Moisture content	14
2.3.5 Culture time	14
3. Purification of fibrinolytic enzymes	14

Contents (continue)

	Page
4. Characterization of fibrinolytic enzymes	17
4.1 Serine proteases	18
4.2 Metalloproteases	20
Objectives	23
2. Materials and Methods	24
Materials	24
1. Microorganism	24
2. Media	24
3. Chemicals	24
Instruments	25
Analytical methods	25
Methods	27
1. Effect of cultural medium and environmental conditions on growth and fibrinolytic enzyme production	27
1.1 Effect of cultural medium	28
1.2 Effect of incubation periods	29
1.3 Effect of pHs	29
1.4 Effect of temperatures	29
1.5 Effect of shaking speed	29
1.6 Time course of growth and fibrinolytic enzyme production under optimal conditions	29
2. Purification of fibrinolytic enzyme	29
2.1 Ammonium sulfate precipitation	29
2.2 Dialysis	30
2.3 Anion-exchange column chromatography	30
2.4 Gel electrophoresis	31

Contents (continue)

	Page
3. Characterization of the partially purified fibrinolytic enzyme	31
3.1 Effect of temperature on enzyme activity	31
3.2 Effect of pH on enzyme stability	32
3.3 Effect of temperature on enzyme stability	32
3.4 Effect of metal ions and chemical reagents on enzyme activity	32
3.5 Enzyme stability after prolonged incubation	33
3. Results and Discussion	34
1. Effect of cultural medium and environmental conditions on growth and fibrinolytic enzyme production	34
1.1 Effect of cultural medium	34
1.2 Effect of incubation periods	36
1.3 Effect of pHs	36
1.4 Effect of temperatures	39
1.5 Effect of shaking speed	41
1.6 Time course of growth and fibrinolytic enzyme production under optimal conditions	41
2. Purification of fibrinolytic enzyme	45
2.1 Ammonium sulfate precipitation	45
2.2 Dialysis	45
2.3 Anion-exchange column chromatography	46
2.4 Gel electrophoresis	50
3. Characterization of the partially purified fibrinolytic enzyme	52
3.1 Effect of temperature on enzyme activity	52
3.2 Effect of pH on enzyme stability	54
3.3 Effect of temperature on enzyme stability	56

Contents (continue)

	Page
3.4 Effect of metal ions and chemical reagents on enzyme activity	58
3.5 Enzyme stability after prolonged incubation	60
4. Conclusion	62
References	64
Appendices	72
Appendix 1 Medium preparation	72
Appendix 2 Buffer preparation	75
Appendix 3 Analytical methods and $(\text{NH}_4)_2\text{SO}_4$ fractionation	79
Appendix 4 Data analysis	87
Publications	90
Vitae	91

List of Tables

Table		Page
1	Fibrinolytic enzymes producing microorganisms	10
2	Composition of medium for cultivation of <i>S. commune</i> BL 23	28
3	Ammonium sulfate precipitation of fibrinolytic activity at various salt saturation	47
4	Effect of ammonium salts and sulfate salts on fibrinolytic activity	47
5	Summary of fibrinolytic enzyme purification steps	48
6	Effect of pH on enzyme stability, the partially purified enzyme of <i>S. commune</i> BL 23 was incubated at room temperature (28°C) for 20 min and 48 h	55
7	Effect of metal ions and chemical reagents on fibrinolytic activity from <i>S. commune</i> BL 23	59
Appendix		
3.1	Experimental set up for the Bradford's method	80
3.2	Final concentrations of ammonium sulfate: percentage saturation	86
4.1	Effect of cultural medium (initial pH = 6) on fibrinolytic enzyme production of <i>S. commune</i> BL 23 cultivated at 30°C, 150 rpm for 7 days	87
4.2	Effect of incubation period on growth and fibrinolytic enzyme production of <i>S. commune</i> BL 23 cultivated in PYGM medium (initial pH = 6.0) at 30°C and 150 rpm	87
4.3	Effect of initial pH of PYGM medium on growth and fibrinolytic enzyme production of <i>S. commune</i> BL 23 cultivated at 30°C, 150 rpm for 7 days	88

List of Tables (continue)

Appendix	Page
4.4 Effect of temperature on growth and fibrinolytic enzyme production of <i>S. commune</i> BL 23 cultivated in PYGM with initial pH of 6.0 at 150 rpm for 7 days	88
4.5 Effect of shaking speed on growth and fibrinolytic enzyme production of <i>S. commune</i> BL 23 cultivated in PYGM with initial pH of 6.0 at 35°C for 7 days	88
4.6 Time course of growth and fibrinolytic enzyme production of <i>S. commune</i> BL 23 cultivated in PYGM medium with initial pH of 6.0 at 35°C and 150 rpm	89

List of Figures

Figure		Page
1	The fruiting body of <i>Schizophyllum commune</i>	3
2	Diagrammatic representation of the fibrinogen molecule and its conversion to the soft clot of fibrin	7
3	Reactions involved in the dissolution of the clot	8
4	Fibrinolytic activity on fibrin plate	27
5	Effect of cultural medium (initial pH = 6) on fibrinolytic enzyme production of <i>S. commune</i> BL 23 cultivated at 30°C, 150 rpm for 7 days	35
6	Effect of incubation time on growth and fibrinolytic enzyme production of <i>S. commune</i> BL 23 cultivated in PYGM medium (initial pH = 6) at 30°C and 150 rpm	37
7	Effect of initial pH of PYGM medium on growth and fibrinolytic enzyme production of <i>S. commune</i> BL 23 cultivated at 30°C, 150 rpm for 7 days	38
8	Effect of temperature on growth and fibrinolytic enzyme production of <i>S. commune</i> BL 23 cultivated in PYGM with initial pH of 6.0 at 150 rpm for 7 days	40
9	Effect of shaking speed on growth and fibrinolytic enzyme production of <i>S. commune</i> BL 23 cultivated in PYGM with initial pH of 6.0 at 35°C for 7 days	42
10	Time course of growth and fibrinolytic enzyme production of <i>S. commune</i> BL 23 cultivated in PYGM medium with initial pH of 6.0 at 35°C and 150 rpm	44
11	Anion exchange column chromatography on DEAE-Sephacel of fibrinolytic enzyme of <i>S. commune</i> BL 23 using linear gradient 0-0.5 M NaCl	49

List of Figures (continue)

Figure	Page
12 Native polyacrylamide gel electrophoresis of protein fractions obtained during purification of fibrinolytic enzyme of <i>S. commune</i> BL 23. Lane 1 standard; 2 crude enzyme; 3, dialysis; 4, DEAE-Sephacel	51
13 Partially purified fibrinolytic enzyme activity on fibrin plate after running native polyacrylamide gel electrophoresis	51
14 Effect of temperature on enzyme activity, the partially purified enzyme of <i>S. commune</i> BL 23 was incubated at various temperature for 18 h	53
15 Effect of temperature on enzyme stability, the partially purified enzyme of <i>S. commune</i> BL 23 in the buffer solution (pH 7.0) was incubated at 40, 50 and 60°C for 48 h.	57
16 The enzyme stability from <i>S. commune</i> BL 23 after prolonged incubation time in the buffer solution (pH 7.0) at 30°C for 60 days.	61
Appendix	Page
3.1 Standard curve of BSA at the absorbance of 750 nm.	81
3.2 Standard curve of BSA at the absorbance of 595 nm.	81

List of Abbreviation

°C	=	Degree celsius
ρCMB	=	ρ-chloromercuribenzoate
μl	=	Microliter
BSA	=	Bovine serum albumin
conc	=	concentration
DEAE	=	Diethylaminoethyl
DFP	=	Diisopropylfluorophosphate
EDTA	=	Ethylenediamine tetraacetic acid
EGTA	=	Ethylene glycol-o-o'-bis [2-amino-ethyl]-N-N-N'-N'-tetraacetic acid
h	=	hours
ICH ₂ COOH	=	Iodoacetic acid
kDa	=	Kilodaltons
l	=	Liter
mg	=	Milligram
min	=	Minute
ml	=	Milliliter
MW	=	Molecular weight
NPGb	=	ρ-nitrophenyl-ρ-guanidobenzoate-HCl
O.D.	=	Optical density
PAGE	=	Polyacrylamide gel electrophoresis
PMSF	=	Phenylmethyl sulfonylfluoride
rpm	=	Revolutions per minute
sat.	=	Saturation
SBTI	=	Soybean trypsin inhibitor
SDS	=	Sodium dodecyl sulfate
TEMED	=	N,N,N',N',-tetramethyl ethylenediamine

List of Abbreviation (continue)

TLCK	=	N-toluenesulfonyl-L-lysine chloromethyl ketone
TPCK	=	N-toluenesulfonyl-L-phenylalanine chloromethyl ketone
U	=	Units
v/v	=	Volumn/volumn