

CONTENTS

	Page
Acknowledgement	c
Abstract in Thai	d
Abstract in English	f
List of Tables	l
List of Figures	n
Chapter 1 Introduction	1
Chapter 2 Literature Review	3
2.1 Mannan	3
2.2 Mannan degrading enzymes	6
2.2.1 Source of β -mannanase	6
2.2.2 β -Mannanase production conditions and properties	9
2.2.3 Applications of β -mannanase	10
2.3 Enzyme purification	18
2.3.1 Protein precipitation	18
2.3.2 Dialysis	19
2.3.3 Ion exchange chromatography	19
2.3.4 Gel filtration chromatography	20
Chapter 3 Materials and Methods	25
3.1 Material and chemical reagents	25
3.1.1 Media and chemical reagents	25
3.1.2 Equipment	26
3.2 Methods	27
3.2.1 Microbial strain and maintenance	27

CONTENTS (CONTINUED)

	Page
3.2.2 Copra meal preparation	27
3.2.3 Enzyme production	27
1) Culture conditions and enzyme production experiment	27
2) Determination of β -mannanase activity	27
3) Determination of protein	28
4) Screening for appropriate nitrogen sources	28
5) Screening for the factors affected on β -mannanase production	28
6) Effect of carbon source on β -mannanase production	29
7) Time course of β -mannanase production	29
8) Effect of growing temperatures on β -mannanase production	33
9) Effect of initial pH on β -mannanase production	33
10) Bioreactor experiment	33
11) Storage stability of the β -mannanase	33
3.2.4 Enzyme purification	34
1) Purification of <i>B. subtilis</i> M7 β -mannanase	34
2) Polyacrylamide gel electrophoresis	34
3.2.5 Characterization of purified β -mannanase	34
1) pH optimum and stability	34
2) Temperature optimum and stability	35
3) Substrate specificity	35
4) Effect of various metal ions and chemicals	35
5) Determination of kinetic parameters	35
6) Hydrolysis experiments	35

CONTENTS (CONTINUED)

	Page
Chapter 4 Results and Discussions	39
4.1 Enzyme production	39
4.1.1 Enzyme production in various nitrogen sources	39
4.1.2 Screening for the factors influenced on β -mannanase production	40
4.1.3 Effect of carbon source on β -mannanase production	42
4.1.4 Time course of β -mannanase production	44
4.1.5 Effect of various temperatures on β -mannanase production	44
4.1.6 Effect of pH on β -mannanase production	44
4.1.7 Scale up β -mannanase production	46
4.1.8 Effect of different additives on stability of β -mannanase	47
4.2 Enzyme purification	48
4.3 Characterization of purified β -mannanase	50
4.3.1 Effects of pH and temperature on enzyme activity	50
4.3.2 Substrate specificity	51
4.3.3 Effect of various metal ions and reagents	52
4.3.4 Kinetics of the β -mannanase reaction	52
4.3.5 Analysis of hydrolysis product	54
Chapter 5 Conclusions	56
References	58
Appendices	68
Appendix A Media	69
Appendix B Measurement activity enzyme	71
Appendix C Measurement protein	73

CONTENTS (CONTINUED)

	Page
Appendix D Electrophoresis	74
Curriculum Vitae	78



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

LIST OF TABLES

	Page
Table 2.1 Typical structures, sources, applications of different subfamilies of mannan polysaccharides	4
Table 2.2 β -Mannanase producing microorganisms	7
Table 2.3 Production conditions and characteristics of mannanases from different microorganisms	11
Table 2.4 Ion exchange groups used in the purification of proteins	20
Table 2.5 Some traditional media for gel filtration of proteins	22
Table 3.1 The concentration of each variable at high and low level used in the Plackett and Burman design while copra meal is a carbon source	30
Table 3.2 Treatments generated based on Plackett and Burman design while copra meal is a carbon source	30
Table 3.3 The concentration of each variable at high and low level used in the Plackett and Burman design while locust bean gum is a carbon source	31
Table 3.4 Treatments generated based on Plackett and Burman design while locust bean gum is a carbon source	31
Table 3.5 The concentration of each variable at high and low level used in the Plackett and Burman design while konjac flour is a carbon source	32
Table 3.6 Treatments generated based on Plackett and Burman design while konjac flour is a carbon source	32
Table 4.1 The least square linear regression analysis of Plackett and Burman statistical design for screening of the important factors affecting on β -mannanase production while copra meal is a carbon source	41
Table 4.2 The least square linear regression analysis of Plackett and Burman statistical design for screening of the important factors affecting on β -mannanase production while locust bean gum is a carbon source	41

LIST OF TABLES (CONTINUED)

	Page
Table 4.3 The least square linear regression analysis of Plackett and Burman statistical design for screening of the important factors affecting on β -mannanase production while konjac mannan is a carbon source	42
Table 4.4 Purification of β -mannanase from <i>B. subtilis</i> M7	49
Table 4.5 Substrate specificity of β -mannanase from <i>B. subtilis</i> M7	52
Table 4.6 Effect of cations and reagents on the activity of β -mannanase from <i>B. subtilis</i> M7	53
Table A.1 Formulation of resolving gel	76
Table A.2 Formulation of stacking gel	76

LIST OF FIGURES

	Page
Figure 3.1 Summary of experimental procedure for β -mannanase production	37
Figure 3.2 Summary of experimental procedure for purification and characterization of β -mannanase	38
Figure 4.1 Effect of inorganic nitrogen source on β -mannanase production by <i>B. subtilis</i> M7 at 37°C with 180 rpm	40
Figure 4.2 Effect of organic nitrogen source on β -mannanase production by <i>B. subtilis</i> M7 at 37°C with 180 rpm	40
Figure 4.3 Effect carbon source of on β -mannanase production by <i>B. subtilis</i> M7. (a) copra meal, (b) locust bean gum and (c) konjac flour	43
Figure 4.4 Time course of β -mannanase production of <i>B. subtilis</i> MR10 (◆) and <i>B. subtilis</i> M7 (▲) cultivated in the optimal medium with (a) copra meal, (b) locust bean gum and (c) konjac flour as a sole carbon source - - -, viable cell (log CFU/ml); —, β -mannanase activity (U/ml)	45
Figure 4.5 Effect of growth temperatures on β -mannanase production by <i>B. subtilis</i> M7 at 180 rpm	46
Figure 4.6 Effect of initial pH on β -mannanase production by <i>B. subtilis</i> M7 at 37°C with 180 rpm	46
Figure 4.7 Time course of β -mannanase production of <i>B. subtilis</i> M7 cultivated in the optimal medium at 37°C with different agitation rates. ◆, 200 rpm; ▲, 400 rpm; - - -, viable cell (log CFU/ml); —, β -mannanase activity (U/ml)	47
Figure 4.8 Storage stability of β -mannanase from <i>B. subtilis</i> M7 with different stabilizers at 4°C	48
Figure 4.9 Elution profiles of β -mannanases from <i>B. subtilis</i> M7 on DEAE-Sephadex A-50 column chromatography pH 7.5	49

LIST OF FIGURES (CONTINUED)

	Page
Figure 4.10 Elution profiles of β -mannanases from <i>B. subtilis</i> M7 on Sephacryl S-100 gel filtration chromatography pH 7.5	49
Figure 4.11 SDS-PAGE of purified β -mannanase from <i>B. subtilis</i> M7. Lane M: molecular weight marker; Lane S: purified β -mannanase	50
Figure 4.12 Effect of pH (a) and temperature (b) on the activity of β -mannanase from <i>B. subtilis</i> M7	51
Figure 4.13 pH stability at 4 °C for 24 (a) and thermostability for 1 h (b) of β -mannanase from <i>B. subtilis</i> M7	51
Figure 4.14 Lineweaver Burk plots of β -mannanase from <i>B. subtilis</i> M7	53
Figure 4.15 Thin layer chromatography of locust bean gum and copra meal hydrolysates digested by the purified β -mannanase from <i>B. subtilis</i> M7 after 6 h incubation. Lane 1, 6 and 11 are standard mannose; Lane 2, 3, 4 and 5 are locust bean gum hydrolysis products from 0, 0.5, 1 and 6 h of reaction time, respectively; Lane 7, 8, 9 and 10 are copra meal hydrolysis products from 0, 0.5, 1 and 6 h of reaction time, respectively	55
Figure A.1 Calibration curve of standard mannose concentration versus the absorbance at 540 nm	72
Figure A.2 Standard curves for protein assay	73