

CATABOLISM OF ACENAPHTHYLENE BY *Rhizobium* sp. CU-A1

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แคแทบอลิซึมของอะซีแนพริลีนโดย *Rhizobium* sp. CU-A1

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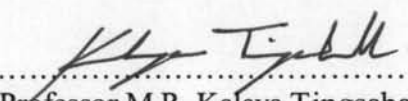
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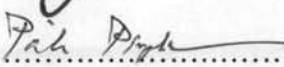
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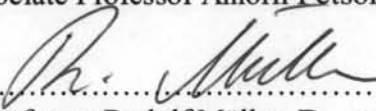

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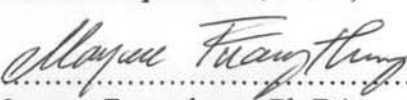

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ศิริวัตร ปุณฺทริกพันธ์ : แคมแทบอลิซึมของอะซีแนพธิลีนโดย *Rhizobium* sp. CU-A1 (CATABOLISM OF ACENAPHTHYLENE BY *Rhizobium* sp. CU-A1) อ. ที่ปรึกษา : รศ.ดร.ไพเราะ ปิ่นพานิชการ, อ. ที่ปรึกษาร่วม : รศ.ดร.อมร เพชรสม, Prof. Dr. rer. nat. Rudolf Müller จำนวนหน้า 170 หน้า. ISBN 974-14-2532-5.

จากรายงานก่อนหน้าถึงวิถีการย่อยสลายอะซีแนพธิลีนใน *Rhizobium* sp. CU-A1 พบว่าสร้าง อะซีแนพธินควิโนน, กรดแนพธาลีน-1,8-ไดคาร์บอกซิลิก และกรดเจนทิลิกเป็นสารมัธยันต์ งานวิจัยนี้ได้ทำการแยกสารมัธยันต์เพิ่มเติมจากส่วนสกัดจากน้ำเลี้ยงเชื้อของสายพันธุ์กลายของสายพันธุ์ CU-A1 ที่ผ่านการกลายพันธุ์ด้วยทรานสไปซอนให้มีความบกพร่องในการย่อยสลายอะซีแนพธิลีน รวมทั้งทำสารมัธยันต์ให้บริสุทธิ์โดยวิธี preparative TLC, ซิลิกาเจลคอลัมน์โครมาโทกราฟี และ HPLC และพิสูจน์เอกลักษณ์ด้วยวิธีแกสโครมาโทกราฟี-แมสสเปกโทเมตรี จากผลการพิสูจน์เอกลักษณ์ของสารมัธยันต์สามารถทำนายวิถีแคมแทบอลิซึมของอะซีแนพธิลีนอย่างสมบูรณ์ใน *Rhizobium* sp. CU-A1 ได้ดังนี้ คือ อะซีแนพธิลีนจะถูกเติมออกซิเจน 2 อะตอมลงบนโมเลกุลของอะซีแนพธิลีนได้เป็นอะซีแนพธินไดออล ซึ่งจะถูกออกซิไดซ์ต่อไปเป็นอะซีแนพธินควิโนน หลังจากนั้นจะถูกเติมออกซิเจนได้เป็นผลิตภัณฑ์จากการแตกวงอะโรมาติกคือกรดแนพธาลีน-1,8-ไดคาร์บอกซิลิก หลังจากนั้นกรดแนพธาลีน-1,8-ไดคาร์บอกซิลิก จะถูกดึงหมู่คาร์บอกซิลบนวงเบนซีนออกหนึ่งหมู่ กลายเป็นสารมัธยันต์ชนิดใหม่คือกรดแนพธอิก ซึ่งจะถูกออกซิไดซ์ต่อไปเป็น 1,2-ไดไฮดรอกซีแนพธาลีน กรดซาลิไซลิก และกรดเจนทิลิก ตามลำดับ หลังจากนั้นจะเกิดการไฮดรอกซิเลชันและการแตกวงอะโรมาติกของกรดเจนทิลิก ได้เป็น สารมัธยันต์ในวัฏจักรเครบส์ โดยผ่านการสร้างมาเลอิลไพรูเวท และ ฟูมาริลไพรูเวท

ส่วนสกัดปราศจากเซลล์ ที่เตรียมได้จากเชื้อนี้เมื่อเลี้ยงในอาหารเหลวเกลือแร่ที่มีกรดโปรโตคาที่คูอิกและกระตุ้นด้วยอะซีแนพธิลีน มีความสามารถในการออกซิไดซ์อะซีแนพธิลีนไปเป็นผลิตภัณฑ์ต่างๆที่สอดคล้องกัน เอนไซม์ อะซีแนพธิลีน ไดออกซิจีเนสที่ทำหน้าที่ในการเร่งปฏิกิริยาขั้นแรกของการย่อยสลายอะซีแนพธิลีน ได้ถูกแยกออกเป็น 3 องค์ประกอบ เรียกว่า ส่วนประกอบ A, B และ C ในระหว่างขั้นตอนการทำเอนไซม์ให้บริสุทธิ์ด้วย เจลฟิลเตรชันโครมาโทกราฟี ส่วนประกอบทั้ง 3 ส่วนมีความจำเป็นร่วมกันในการแสดงแอกติวิตีและได้ผลิตภัณฑ์เป็นอะซีแนพธินไดออล ส่วนประกอบ A คือเทอริมินัล ออกซิจีเนส ประกอบด้วยหน่วยย่อย 2 หน่วยที่มีน้ำหนักโมเลกุลไม่เท่ากัน คือ 45 และ 22 กิโลดาลตัน ส่วนประกอบ B และ ส่วนประกอบ C คือ รีดักเทส และ เฟอร์รีดอกซิน ที่มีน้ำหนักโมเลกุลเท่ากับ 48.2 และ 9.8 กิโลดาลตัน ตามลำดับ สเปกตรัมของการดูดกลืนแสงยูวี/วิซิเบิลของส่วนประกอบ A และ C มีลักษณะเช่นเดียวกับโปรตีนที่มี Rieske type iron-sulfur center ขณะที่ยูวี/วิซิเบิลสเปกตรัมของส่วนประกอบ B เป็นลักษณะของฟลาโวโปรตีน ดังนั้นในเบื้องต้นอะซีแนพธิลีน ไดออกซิจีเนส ของ *Rhizobium* sp. CU-A1 ถูกจัดให้อยู่ในไดออกซิจีเนสกลุ่ม IIB ที่ทำหน้าที่ในการเร่งปฏิกิริยาการเติมออกซิเจนและการแตกวงอะโรมาติกของสารตั้งต้น

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KEY WORD: *Rhizobium* sp./ ACENAPHTHYLENE/ ACENAPHTHENEQUINONE/
NAPHTHALENE-1,8-DICARBOXYIC ACID/ 1-NAPHTHOIC ACID/ GENTISIC ACID/
DIOXYGENASE

SIRIWAT POONTHRIGPUN : CATABOLISM OF ACENAPHTHYLENE BY *Rhizobium* sp.
CU-A1 THESIS ADVISOR : ASSOC. PROF. PAIROH PINPANICHAKARN, PH.D.,
THESIS COADVISOR : ASSOC. PROF. AMORN PETSOM, PH.D., PROF. RUDOLF
MÜLLER, DR. RER. NAT., 170 pp. ISBN 974-14-2532-5.

Rhizobium sp. CU-A1 was previously shown to mineralize acenaphthylene via the formation of acenaphthenequinone, naphthalene-1,8-dicarboxylic acid and gentisic acid as the intermediates. In this work, several additional intermediates were isolated from the extracts of the culture broth of its blocked mutants, purified by thin-layer, silica gel column or high-performance liquid chromatography and identified by gas chromatography-mass spectrometry. As a result, a complete pathway for the catabolism of acenaphthylene by *Rhizobium* sp. CU-A1 is proposed as follow: the initial reaction is the incorporation of two oxygen atoms into acenaphthylene molecule to form acenaphthenediol which then further oxidized to acenaphthenequinone. Subsequent dioxygenation would yield a ring fission product, naphthalene-1,8-dicarboxylic acid. A possible decarboxylation would give a novel intermediate, 1-naphthoic acid, which would further be oxidized to 1,2-dihydroxynaphthalene, salicylic acid and gentisic acid, respectively. Then, hydroxylation of gentisic acid, following by ring cleavage, would give central intermediates in the TCA cycle via maleylpyruvate or fumarylpyruvate formation.

Crude cell free extract prepared from this organism grown in mineral medium supplemented with protocatechuic acid and induced by acenaphthylene showed ability to oxidize acenaphthylene to the corresponding oxidation products. The enzyme catalyzing the initial reaction of acenaphthylene degradation, acenaphthylene dioxygenase, was separated into three components, designated as components A, B, and C by gel filtration chromatography. All three components were essentially required for the activity giving acenaphthenediol as a product. Component A, terminal oxygenase, was a dimer with two subunits with molecular mass of 45 and 22 kDa. Component B, reductase, and C, ferridoxin, consisted of a single polypeptide with a molecular mass of 48.2 and 9.8 kDa, respectively. Component A and C had UV/visible spectra of a Rieske type iron-sulfur center while component B had a UV/vivable spectrum of flavoprotein. Acenaphthylene dioxygenase from *Rhizobium* sp. CU-A1 was presumed to be a class IIB dioxygenase which had ring hydroxylating and ring fission activity.

Student's Signature..... 

Field of Study Environmental Management...Advisor's Signature..... 

Academic Year 2006..... Co-advisor's Signature..... 

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CONTENTS

	Pages
Abstract in Thai.....	IV
Abstract in English.....	V
Acknowledgements.....	VI
Contents.....	VII
List of Tables.....	VIII
List of Figures.....	X
Chapter	
1. Introduction.....	1
2. Literature Reviews.....	5
3. Materials and Methods.....	30
4. Results and Discussion.....	51
5. Conclusion.....	128
References.....	135
Appendices	
Appendix A.....	155
Appendix B.....	157
Appendix C.....	165
Biography.....	170

LIST OF TABLES

Table	Pages
2.1	Properties of some aromatic dioxygenases.....25
4.1	Summary of the acenaphthylene intermediates.....67
4.2	Products formation from the transformation of various compounds by <i>Rhizobium</i> sp. CU-A1 and its transposon mutants.....70
4.3	HPLC retention times and electron impact mass spectral properties of the intermediates from acenaphthylene degradation by crude cell free extract of <i>Rhizobium</i> sp. CU-A1.....86
4.4	Catalytic activity of the crude cell free extract on the intermediates from acenaphthylene degradation. Products formed were identified by cochromatography with authentic compounds.....88
4.5	Effect of various supplements on acenaphthylene dioxygenase activity in the cell extract.....93
4.6	Fractionation of acenaphthylene dioxygenase by ammonium sulfate precipitation.....96
4.7	Resolution of acenaphthylene dioxygenase into three protein components by gel filtration on Superdex G200.....99
4.8	Reconstitution of components A, B and C for acenaphthylene dioxygenase activity.....100
4.9	Cytochrome <i>c</i> reductase activity of components A, B and C.....102
4.10	Summary of the purification of acenaphthylene dioxygenase.....103
4.11	Purification table of component A.....106
4.12	Purification table of component B from gel filtration.....106
4.13	Classification of dioxygenase.....117
4.14	Activities of acenaphthylene dioxygenase on different compounds.....121
4.15	Effects of metal ions and chemicals on acenaphthylene dioxygenase activity.....126
5.1	Properties of acenaphthylene dioxygenase enzyme complex from <i>Rhizobium</i> sp. CU-A1.....131

LIST OF TABLES (CONT)

Table		Pages
A.1	<i>Rhizobium</i> sp. CU-A1 transposon mutants used in this study and its ability to grow on carbon sources (Kriengkripipat, 2001).....	156
B.1	Scheme for the preparation of 12% Native gel.....	162
B.2	Scheme for the preparation of 12% SDS gel.....	163
C.1	Standard proteins and their K_{av} values.....	168
C.2	Standard proteins and their mobility in SDS-PAGE.....	169

LIST OF FIGURES

Figure	Pages
2.1	Molecular structure of acenaphthylene.....6
2.2	Catabolic pathway for the degradation of naphthalene by <i>Sphingomonas yanoikuyae</i> B1 and <i>Pseudomonas putida</i> G7 (Kim et al., 1997; Yen and Gunsulas, 1982).....9
2.3	Salicylic acid catabolic pathway via catechol in <i>Pseudomonas putida</i> and <i>Pseudomonas</i> sp. ND-6 (Meer et al., 1992 and Zhao et al. 2005).....10
2.4	Salicylic acid catabolic pathway in <i>Ralstonia</i> U2 (Ning et al., 2001 and 2002).....11
2.5	Initial step of catabolic pathways proposed for the metabolism of pyrene and benzo[<i>a</i>]pyrene by bacteria.....12
2.6	Acenaphthylene catabolic pathway by <i>Trametes versicolor</i> (Johannes et al., 1998).....15
2.7	Proposed pathway for the oxidation of acenaphthylene by <i>Pseudomonas aeruginosa</i> PA01 (Selifenov et al., 1996).....17
2.8	Oxidation of naphthalene by multicomponent enzyme system, naphthalene dioxygenase from <i>Pseudomonas putida</i> NCIB 9816 (Haigler and Gibson, 1990; Suen and Gibson, 1993).....22
2.9	Catalytic cycle of naphthalene-1,2-dioxygenase (Bassan et al., 2004).....23
3.1	Acenaphthylene dioxygenase purification scheme.....46
4.1	Growth and acenaphthylene utilization of mutants A53, E11 and B1 in mineral medium containing 600 mg/L acenaphthylene comparing with <i>Rhizobium</i> sp. CU-A1.....52
4.2	TLC chromatograms of the culture extracts showing intermediates produced from the degradation of acenaphthylene in day 0, 4, 8 and 12 by mutants A53, E11 and B1 grown in mineral medium containing 600 mg/L acenaphthylene comparing with <i>Rhizobium</i> sp. CU-A1. (AE, acenaphthylene; w/o, medium without bacteria). The arrow indicates the intermediate with R _f value 0.53 giving strong UV intensity on Day 4 in mutant A53.....53

LIST OF FIGURES (CONT)

Figure	Pages
4.3 HPLC chromatograms of the culture extracts showing intermediates produced from the degradation of acenaphthylene in day 0 and 12 by mutants A53, E11 and B1 grown in mineral medium containing 600 mg/L acenaphthylene comparing with <i>Rhizobium</i> sp. CU-A1.....	54
4.4 TLC chromatogram of each fraction of acenaphthylene degrading intermediates from preliminary purified A53-culture extract eluted from silica gel column chromatography with 0-100% ethyl acetate in hexane.....	55
4.5 HPLC chromatogram of each fraction of acenaphthylene degrading intermediates from preliminary purified A53-culture extract eluted from silica gel column chromatography with 0-100% ethyl acetate in hexane.....	56
4.6 Mass spectrum of intermediate I from A53 [A] compared with authentic standard 1-naphthoic acid [B].	57
4.7 Molecular structure of 1-naphthoic acid.....	57
4.8 HPLC chromatograms of E11-culture extracts showing intermediates produced from the degradation of acenaphthylene in day 0, 6 and 14 of incubation. The arrows indicate a predominant intermediate with retention time of 23.5 min to be identified.....	58
4.9 HPLC chromatograms of the partially purified intermediate II (A) compared with the culture extract of E11 (B).....	59
4.10 Mass spectrum of intermediate II from E11 [A] compared with authentic standard 1,2-dihydroxynaphthalene [B].....	59
4.11 Molecular structure of 1,2-dihydroxynaphthalene.....	60
4.12 HPLC chromatograms of intermediates produced from the degradation of acenaphthylene by B1 after 8, 12 and 18 h of incubation. The arrow indicates an intermediate with retention time of 25.7 min to be identified.....	61
4.13 HPLC chromatograms of purified intermediate III (A) compared with the culture extract of B1 (B).....	61
4.14 Mass spectrum of intermediate III from B1 [A] compared with those of 1,2-acenaphthenediol found in MS database [B].....	62

LIST OF FIGURES (CONT)

Figure	Pages
4.15	Molecular structure of 1,2-acenaphthenediol.....62
4.16	HPLC elution profile of the extract from the culture of <i>Rhizobium</i> sp. CU-A1 cultivated in mineral medium for 48 h in the presence of acenaphthylene. The arrow indicates an intermediate with retention time of 19.784 min to be identified.....63
4.17	Mass spectrum of intermediate IV from <i>Rhizobium</i> sp. CU-A1 [A] compared with those of 3-hydroxybenzoic acid and salicylic acid found in MS database and those of authentic standard salicylic acid and 3-hydroxybenzoic acid [B].....64
4.18	Growth profiles of <i>Rhizobium</i> sp. CU-A1 on salicylic acid (A) and 3-hydroxybenzoic acid.....65
4.19	Molecular structure of salicylic acid (A) and 3-hydroxybenzoic acid (B).....65
4.20	GC chromatogram of the culture extract of <i>Rhizobium</i> sp. CU-A1. [A] and mass spectrum of the compounds, eluted at retention time between 10.7-10.9 min [B].....66
4.21	Molecular structure of maleylpyruvate [A] and fumarylpyruvate [B].....66
4.22	Growth profiles of <i>Rhizobium</i> sp. CU-A1 in mineral medium containing 300 mg/L acenaphthenequinone (1), naphthalene-1,8-dicarboxylic acid (2), 1-naphthoic acid (3), 1,2-dihydroxynaphthalene (4), salicylic acid (5) and gentisic acid (6).....71
4.23	Growth profiles of A53 in mineral medium containing 300 mg/L acenaphthenequinone (1), naphthalene-1,8-dicarboxylic acid (2), 1-naphthoic acid (3), 1,2-dihydroxynaphthalene (4), salicylic acid (5) and gentisic acid (6).....72
4.24	Growth profiles of B1 in mineral medium containing 300 mg/L acenaphthenequinone (1), naphthalene-1,8-dicarboxylic acid (2), 1-naphthoic acid (3), 1,2-dihydroxynaphthalene (4), salicylic acid (5) and gentisic acid (6).....73

LIST OF FIGURES (CONT)

Figure	Pages
4.25 Growth profiles of E11 in mineral medium containing 300 mg/L acenaphthenequinone (1), naphthalene-1,8-dicarboxylic acid (2), 1-naphthoic acid (3), 1,2-dihydroxynaphthalene (4), salicylic acid (5) and gentisic acid (6).....	74
4.26 The disappearance of acenaphthylene and the appearance of intermediates during the growth of <i>Rhizobium</i> sp. CU-A1 in mineral medium containing acenaphthylene; ●: number of cell, □: acenaphthylene, ■: acenaphthenequinone, △: naphthalene-1,8-dicarboxylic acid, ▲: 1-naphthoic acid, ◇: 1,2-dihydroxynaphthalene, ◆: salicylic acid, ○: gentisic acid.....	75
4.27 HPLC elution profile of intermediates formed from the degradation of acenaphthylene by <i>Rhizobium</i> sp. CU-A1 after 36 h of cultivation.....	76
4.28 Acenaphthylene catabolic pathway in <i>Beijerinckia</i> sp. (Shocken and Gibson, 1984) and <i>Pseudomonas aeruginosa</i> PA01 (Selifenov et al., 1996) (A) and a proposed acenaphthylene catabolic upper pathway in <i>Rhizobium</i> sp. CU-A1 (B), (The red color represents the isolated and identified intermediate from this work).....	77
4.29 Pyrene catabolic pathway in <i>Mycobacterium</i> sp. PYR-1 (Young et al., 1995) <i>Mycobacterium</i> sp. KR2 (Rehmann et al., 1998) and <i>Mycobacterium</i> sp. AP1 (Vila et al., 2001) (A) and a proposed naphthalene-1,8-dicarboxylic acid decarboxylation to 1-naphthoic acid in <i>Rhizobium</i> sp. CU-A1 (B), (The red color represents the isolated and identified intermediate from this work).....	79
4.30 Proposed 1-naphthoic acid catabolic pathway in <i>Rhizobium</i> sp. CU-A1 (A) and 1-naphthoic acid catabolic pathway in <i>Pseudomonas maltophilia</i> CSV89 (Phale et al.,1995) (B), (The red color represents the isolated and identified intermediates from this work; I, 1,2-dihydroxy-8-carboxynaphthalene; II, 2-carboxy-2-hydroxy-8-carboxychromene; III, 2-hydroxy-3-carboxybenzyl pyruvate; IV, 3-formyl-salicylic acid; V, 2-hydroxyisophthalic acid).....	80

LIST OF FIGURES (CONT)

Figure	Pages
4.31	Salicylic acid catabolic pathway via gentisic acid in <i>Ralstonia</i> U2 (Ning et al., 2001 and 2002) (A) and proposed salicylic acid catabolic pathway in <i>Rhizobium</i> sp. CU-A1 (B), (The red color represents the isolated and identified intermediates from this work).....81
4.32	Proposed acenaphthylene catabolic pathway in <i>Rhizobium</i> sp. CU-A1.....83
4.33	Oxidation profiles of acenaphthylene by crude cell free extract (●), boiled cell free extract(◆) and without crude cell free extract (■).....84
4.34	Oxidation profiles of acenaphthenequinone (A), naphthalene-1,8-dicarboxylic acid (B), 1-naphthoic acid (C), 1,2-dihydroxynaphthalene (D), salicylic acid (E), and gentisic acid (F) by crude cell free extract (●), boiled cell free extract(◆) and without crude cell free extract (■).....85
4.35	HPLC elution profiles of acenaphthylene oxidation products produced from the reaction with the crude cell free extract from <i>Rhizobium</i> sp. CU-A1 when incubated at 35°C for 0 h (A), 1 h (B), 2 h (C), and 3 h (D). I to VI are oxidation products after 2 h of incubation.....87
4.36	Effect of N-ethylmaleimide, a sulfhydryl agent, on the enzyme activity using acenaphthylene as a substrate.....89
4.37	Effect of dithiothreitol to restore an enzyme activity after treatment with 0.2 mM N-ethylmaleimide.....90
4.38	Effect of dithiothreitol concentrations on stabilization of the enzyme activity.91
4.39	Effect of dithiothreitol concentrations on the enzyme activity.....92
4.40	Effect of pH [A] and temperature [B] on the enzyme activity in the cell free extract.....92
4.41	Dependence of the enzyme activity on protein concentration in the crude cell free extract.....94
4.42	Effect of NaCl and KCl on acenaphthylene oxidizing activity of the crude cell free extract.....95

LIST OF FIGURES (CONT)

Figure	Pages
4.43	Anion exchange chromatography using Q Sepharose fast flow column of 40-60% salt saturated fraction. Protein as determined by optical density at 280 nm.....98
4.44	Superdex G200 chromatography of acenaphthylene oxidizing protein from ion exchange chromatography represents resolution of the enzyme into three protein components, A (brown), B (yellow) and C (light brown). Protein was determined by optical density at 280 nm and enzyme activity was assayed as described in 3.5.3 using 100 μ l of the fraction to be determined in the presence of 25 μ g/ml each of the other two components.....99
4.45	GC chromatogram (A) and mass spectrum (B) of the oxidation product (indicated by an arrow in A) of acenaphthylene compare with the mass spectrum of 1,2-acenaphthenediol (C).....101
4.46	Hydroxyapatite chromatography of the component A.....104
4.47	Chromatography of component A on phenyl Sepharose column.....105
4.48	Blue Sepharose chromatography of component B from gel filtration fraction.....107
4.49	PAGE of samples from each step of the enzyme purification taken during the purification. Lanes: 1 crude cell extract; 2, 40-60% $(\text{NH}_4)_2\text{SO}_4$ fraction; 3, Q Sepharose active fraction; 4, Component A from Superdex G200 column; 5, Component A from hydroxyapatite column; 6, Component A from phenyl Sepharose column; 7, Component B from Blue Sepharose column; 8, Component C from Superdex G200 column.....109
4.50	SDS-PAGE of samples from each step of the enzyme purification. taken during the purification. Lanes: 1 crude cell extract; 2, 40-60% $(\text{NH}_4)_2\text{SO}_4$ fraction; 3, Q Sepharose active fraction; 4, Component A from Superdex G200 column; 5, Component A from hydroxyapatite column; 6, Component A from phenyl Sepharose column; 7, low molecular weight markers 8, Component B from Superdex G200 column; 9, Component B from Blue Sepharose column; 10, Component C from Superdex G200 column.....111

LIST OF FIGURES (CONT)

Figure	Pages
4.51 Absorption spectrum of component A.....	112
4.52 Absorption spectrum of component B.....	113
4.53 Absorption spectrum of component C.....	113
4.54 The proposed electron transport scheme for acenaphthylene dioxygenase from <i>Rhizobium</i> sp. CU-A1.....	118
4.55 Effect of temperature and pH on the activity of acenaphthylene dioxygenase complex protein.....	118
4.56 Temperature and pH stability of acenaphthylene dioxygenase enzyme complex.....	119
4.57 Temperature and pH on stability of component A (●), B (◆) and C (■).....	120
4.58 Lineweaver-Burk plot of acenaphthylene dioxygenase for acenaphthylene....	122
4.59 Mass spectra of acenaphthenediol, the product produced from the oxidation of acenaphthylene by acenaphthylene dioxygenase enzyme system in the presence of only $^{16}\text{O}_2$ (A) and in the presence of both $^{16}\text{O}_2$ and $^{18}\text{O}_2$ (B).....	123
4.60 Mass spectra of naphthalic anhydride (naphthalene-1,8-dicarboxylic acid), the product produced from the oxidation of acenaphthenequinone by acenaphthylene dioxygenase enzyme system in the presence of only $^{16}\text{O}_2$ (A) and in the presence of both $^{16}\text{O}_2$ and $^{18}\text{O}_2$ (B).....	124
4.61 The catalytic activities of acenaphthylene dioxygenase on the oxidation of acenaphthylene to acenaphthenediol and acenaphthenequinone to naphthalene-1,8-dicarboxylic acid in the acenaphthylene catabolic pathway.....	125
C.1 Standard curve of acenaphthylene generated by plotting of acenaphthylene concentration (mg/l) and peak area analyzed by HPLC.....	165
C.2 Standard curve of acenaphthylene generated by plotting of acenaphthylene concentration (nmole) and peak area analyzed by HPLC.....	166
C.3 Standard curve of Bovine serum albumin for determination of protein concentration.....	167

LIST OF FIGURES (CONT)

Figure		Pages
C.4	Calibration curve for determination of molecular mass of proteins by gel filtration chromatography.....	168
C.5	Calibration curve for determination of molecular mass of proteins by SDS-PAGE.....	169