Contents

		Page
บทคัดย่อ		(3)
Abstract		(5)
Acknowled	gement	(7)
Contents		(8)
List of Tab	les	(11)
List of Figu	ures	(13)
Abbreviations and Symbols		(17)
Chapter		
1. Introdu	ction	1
Lite	erature review	2
1.	Phototropic Bacteria	2
2.	Synthesis Pathway of 5-Aminolevulinic Acid	4
	2.1 The Shemin Pathway (C ₄ Pathway)	4
	2.2 The Beale Pathway (C ₅ Pathway)	6
3.	Enzymes Relating to 5-Aminolevulinic Acid Production	7
4.	Regulation of 5-Aminolevulinic Acid Production	12
	4.1 Carbon and nitrogen sources	12
	4.2 Precursors	12
	4.3 Inhibition of ALA dehydratase	13
	4.4 Metal ions	13
	4.5 Light intensity	14
	4.6 pH	14
5.	Strain Improvement	15
Obi	ectives	17
_	als and Methods	18
	terials	18
	alytical Methods	19

Contents (Continued)

		Page
M	ethods	22
1.	Mutagenesis and Screening on Mutant Producing	
	the Highest 5-Aminolevulinic Acid (ALA) Concentration	22
	1.1 Strain improvement by mutagenesis	22
	1.1.1 Mutagenesis by UV	22
	1.1.2 Mutagenesis by NTG	22
	1.2 Screening for the highest ALA-producing mutant strain	22
2.	Optimization for ALA Production by the Selected	
	Mutant Strain	23
	2.1 Effect of using glucose instead of malate	24
	2.2 Effect of levulinic acid (LA) concentration and	
	number of repeated addition	24
	2.3 Effect on the addition of C ₅ pathway precursor	24
	2.4 Effect on the addition of C ₄ pathway precursor	25
	2.5 Effect of C ₄ and C ₅ pathway precursors and their mixture	26
	2.6 Effect of initial pH	26
	2.7 Effect of volatile fatty acids	26
	2.8 Effect of MgCl ₂ .6H ₂ O	26
	2.9 Effect of pyridoxal phosphate	26
	2.10 Effect of controlling pH	27
	2.11 Effect of NaCl concentration	27
3.	Comparison between analytical grade medium and commercial	
	grade medium for 5-aminolevulinic acid production	27
Re	esults and Discussions	29
1.	Mutagenesis and Screening Method on Mutant Producing	
	the Highest ALA Concentration	29

3.

Contents (Continued)

	Page
2. Optimization for ALA Production by the Selected Mutant Strain	
2.1 Effect of using glucose instead of malate	44
2.2 Effect of levulinic acid (LA) concentration and	
number of repeated addition	48
2.3 Effect on the addition of C ₅ pathway precursor	55
2.4 Effect on the addition of C ₄ pathway precursor	56
2.5 Effect of initial pH	65
2.6 Effect of volatile fatty acids	67
2.7 Effect of MgCl ₂ .6H ₂ O	71
2.8 Effect of pyridoxal phosphate	73
2.9 Effect of controlling pH	73
2.10 Effect of NaCl concentration	76
3. Comparison between analytical grade medium and commercial grade	
medium for 5-aminolevulinic acid production	78
4. Conclusions	82
Suggestions	85
References	86
Appendix	92
Vitae	97

List of Tables

Table		
1	Comparison on ALA production from UV mutant strain of	
	Rhodobacter sphaeroides U7 after 48 h cultivation in GM	
	medium under aerobic-dark condition at 37°C	30
2	Comparison on ALA production from UV mutant strain	
	of Rhodobacter sphaeroides N20 after 48 h cultivation	
	in GM medium under aerobic-dark condition at 37 C	
	31	
3	Comparison on ALA production from UV mutant strain	
	of Rhodobacter capsulatus SS3 after 48 h cultivation	
	in GM medium under aerobic-dark condition at 37 \mathbb{I} \text{ C}	
	32	
4	Comparison on ALA production from NTG mutant strain	
	of Rhodobacter sphaeroides U7 after 48 h cultivation	
	in GM medium under aerobic-dark condition at 370 C	
	33	
5	Comparison on ALA production from NTG mutant strain	
	of Rhodobacter sphaeroides N20 after 48 h cultivation	
	in GM medium under aerobic-dark condition at 370 C	
	34	
6	Comparison on ALA production from NTG mutant strain	
	of Rhodobacter capsulatus SS3 after 48 h cultivation	
	in GM medium under aerobic-dark condition at 37 \(\text{\baracle} \) C	
	35	
7	Kinetic parameters from each mutant strain in GM medium	
	under aerobic-dark condition	36
8	Kinetic parameters of growth and ALA production of the	
	selected mutant (Rhodobacter capsulatus SN28) compared	
	to the wild type strain (Rhodobacter capsulatus SS3) in GM	

	List of Tables (Continued)	
Tab	ble	Page
10	Activity of enzyme ALA synthase (ALAS) and ALA	
	dehydratase (ALAD) from Rhodobacter capsulatus SS3	
	and R. capsulatus SN28 cultivated in GG medium+3% NaCl	
	under aerobic-dark condition at 37 \(\text{C} \) 50	
11	Effect of levulinic acid addition on activity of enzyme ALA	
	synthetase and ALA dehydratase from Rhodobacter capsulatus	
	SN28 in GG+3% NaCl under aerobic-dark condition at 37 \(\text{C} \)	
12	Comparison on kinetic parameters of Rhodobacter capsulatus	
	SN28 during cultivation in analytical and commercial	
	grade medium	80
13	Summary on the effect of various parameters on growth and	
	ALA production from Rhodobacter capsulatus SN28 cultivated	
	in GG medium+3%NaCl under aerobic-dark condition at 37°C	84

medium under aerobic-dark condition at 37 \mathbb{I} C

and ALA production of Rhodobacter capsulatus SN28

Effect of medium formular (GM and GG medium) on growth

40

9

47

List of Figures

Figure		Page
1	Tetrapyrrole biosynthesis	5
2	Scheme of the C5-pathway	6
3	Reaction catalysed by ALA synthase. The enzyme catalyses	
	the condensation of glycine and succinyl-CoA in a reaction	
	that requires pyridoxal phosphate PLP as a cofactor. Carbon	
	dioxide, CoA and ALA are generated during the reaction	8
4	Two molecules of 5-ALA condense to porphobilinogen	9
5	Scheme of the C5-pathway	10
6	Growth of the NTG mutant Rhodobacter capsulatus SN28 and	
	the wild-type Rhodobacter capsulatus SS3 in GM medium under	
	aerobic-dark condition at 37°C	38
7	Extracellular ALA production of the NTG mutant Rhodobacter	
	capsulatus SN28 and the wild-type R. capsulatus SS3 in GM	
	medium under aerobic-dark condition at 37°C	39
8	SEM images of the mutant strain Rhodobacter capsulatus SN28	41
9	TEM images of the mutant strain Rhodobacter capsulatus SN28	42
10	TEM images of the wild type Rhodobacter capsulatus SS3	43
11	pH and growth during cultivation of the Rhodobacter capsulatus	
	SN28 in glutamate-malate (GM) medium and glutamate-glucose	
	(GG) medium under aerobic-dark condition at 37°C	45

12	Extracellular ALA production during cultivation of the <i>Rhodobacter</i>	
	capsulatus SN28 in glutamate-malate (GM) medium and glutamate	
	-glucose (GG) medium under aerobic-dark condition at 37°C	46
13	Effect of levulinic acid concentrations on pH, growth and	
	extracellular ALA formation during cultivation of Rhodobacter	
	capsulatus SN28 under aerobic-dark condition at 37° C in GG	
	medium with the addition of levulinic acid (LA) at 36 h	49
	List of Figures (Continued)	
Figure		Page
14	Effect of repeated addition of levulinic acid on pH and	
	growth during cultivation of the mutant Rhodobacter	
	capsulatus SN28 in GG medium under aerobic-dark	
	condition at 37°C	53
15	Effect of repeated addition of levulinic acid on extracellular	
	ALA formation during cultivation of Rhodobacter capsulatus	
	SN28 in GG medium under aerobic-dark condition at 37°C	54
16	Effect of supplementing C5 pathway precursors	
	(glutamate and malic acid) on pH during cultivation of the	
	mutant Rhodobacter capsulatus SN28 under aerobic-dark condition	
	at 37°C	57
17	Effect of supplementing C5 pathway precursors	
	(glutamate and malic acid) on growth during cultivation of the	
	mutant Rhodobacter capsulatus SN28 under aerobic-dark condition	
	at 37°C	58
18	Effect of supplementing C5 pathway precursors	
	(glutamate and malic acid) on extracellular ALA formation during	
	cultivation of the mutant Rhodobacter capsulatus SN28 under	
	aerobic-dark condition at 37°C	59
19	Comparison on pH, growth and extracellular ALA production	

	during cultivation of the mutant Rhodobacter capsulatus SN28	
	in GG medium (no glutamate) with and without addition of LA	
	at 36 h under aerobic-dark condition at 37°C	60
20	Effect of supplementing C4 pathway precursors (glycine and succinate)	
	on pH during cultivation of the mutant Rhodobacter capsulatus SN28 in	GG
	medium under aerobic-dark condition at 37°C	62

List of Figures (Continued)

Figure		Page
21	Effect of supplementing C4 pathway precursors	
	(glycine and succinate) on growth during cultivation of the	
	mutant Rhodobacter capsulatus SN28 in GG medium under	
	aerobic-dark condition at 37°C	63
22	Effect of supplementing C4 pathway precursors	
	(glycine and succinate) on extracellular ALA formation during	
	cultivation of the mutant Rhodobacter capsulatus SN28 in GG	
	medium under aerobic-dark condition at 37°C	64
23	Effect of initial pH on pH, growth and extracellular ALA	
	formation during cultivation of the mutant Rhodobacter	
	capsulatus SN28 in GG medium under aerobic-dark condition	
	at 37°C	66
24	Effect of volatile fatty acid on pH during cultivation of <i>Rhodobacter</i>	
	capsulatus SN28 in GG medium under aerobic-dark condition at 37°C	68
25	Effect of volatile fatty acid on growth during cultivation of <i>Rhodobacter</i>	
	capsulatus SN28 in GG medium under aerobic-dark condition at 37°C	69
26	Effect of volatile fatty acid on extracellular ALA formation during	
	cultivation of Rhodobacter capsulatus SN28 in GG medium under	
	aerobic-dark condition at 37°C	70
27	Effect of MgCl ₂ on pH, growth and extracellular ALA	

	formation during cultivation of Rhodobacter capsulatus	
	SN28 in GG medium under aerobic-dark condition at 37°C	72
28	Effect of pyridoxal phosphate on pH, growth and extracellular	
	ALA formation during cultivation of Rhodobacter capsulatus	
	SN28 in GG medium under aerobic-dark condition at 37°C	74
29	Effect of controlling pH on pH, growth and extracellular ALA	
	formation during cultivation of Rhodobacter capsulatus SN28	
	in GG medium under aerobic-dark condition at 37°C	75
	List of Figures (Continued)	
Figur	re	Page
30	Effect of NaCl concentrations on pH, growth and extracellular	
	ALA formation during cultivation of Rhodobacter capsulatus	
	SN28 in GG medium under aerobic-dark condition at 37°C	77
31	Effect of different medium on pH, growth and extracellular	
	ALA formation during cultivation of Rhodobacter capsulatus	
	SN28 under aerobic-dark condition at 37°C	79

Abbreviations and Symbols

 \mathbb{I} C = degree Celsius

conc = concentration

g = gram

x g = gravitational force

h = hour L = litre

mg/l = millgram per litre

min = minute
ml = milliliter
mM = millimolar
nm = nanometer

OD = optical density

Rm = productivity rate

rpm = round per minute

s = second

w/v = weight by volume w/w = weight by weight

% = percent

g/mol = gram per molar

vvm = volume of air per volume of medium per minute