Chapter 4

Conclusions

1. Among 323 mutant strains of the halotolerant photosynthetic bacterium *Rhodobacter capsulatus* SS3 and the mutant strains of *Rhodobacter sulfidophilus* ES16 (N20, U7), the mutant strain SN28 gave the highest value of extracellular ALA (64.77μM) in GM medium.
2. Comparison on using GM and GG medium, it was found that GG medium gave higher extracellular ALA (97.13 μM) than GM medium (64.77 μM).
3. Optimization for ALA production from the mutant strain SN28 gave the following results:

    3.1 The optimum concentration and number of addition of levulinic acid (LA) for ALA production was at 15 mM, 1 time addition at 36 h after cultivation which gave 129.4 μM.

    3.2 ALA precursors for C5 pathway (glutamate and malic acid) had no effect on ALA production and without glutamate (modified GG medium), the extracellular ALA further increased to 246.6 μM.

    3.3 ALA precursors for C4 pathway (succinate and glycine) had no effect on ALA production.

    3.4 The optimum initial pH was 7.0.

    3.5 The optimum type and the concentration of volatile fatty acid (acetic, propionic and butyric acids) were 0.5 g/l butyric acid which gave the highest ALA production of 267.4 μM.

    3.6 The optimum concentration of $MgCl_2$ for ALA production was at 15 mM which gave the highest ALA production of 303.13 μM.

    3.7 Controlling during cultivation of *R. capsulatus* SN28 gave the maximum ALA concentration of 107.6 μM).

    3.8 Addition of pyridoxal phosphate did not enhance ALA production of *R. capsulatus* SN28.

3.9 The optimum concentration of NaCl for ALA production was at 2% NaCl which gave the highest ALA production of 308.72 μM.

3.10 Comparison on cultivation of the mutant strain SN28 in the analytical grade medium (GM, GG and optimized medium) and commercial grade medium (MGG, MGS and MGSY medium), the analytical grade resulted in the maximum values of extracellular ALA (310.54 μM).

Table 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

Parameter	Conc.	Optimum conc	ALA (μM)	Production rate (μM/h)
glucose instead of malate		glucose	97.13	1.62
LA	5-20 mM	15 mM	129.37	2.16
Repeated addition of LA	1-3 times	1 time	173.09	2.88
C5 pathway precursors	13-55 mM glutamate & 7-30 mM malic	no glutamate without glutamate & LA	246.57	4.11
C4 pathway precursors	2.5-10 mM glycine 10-40 mM succinate	-	-	-
Initial pH	5.5-8.0	7.0	267.92	4.47
Acetic, propionic & butyric acid	0.5-3 g/l	0.5 g/l Butyric acid	267.36	4.47
MgCl2	5-20 mM	15 mM	303.13	5.05
Pyridoxal phosphate	10-40 μM	-	-	-
Controlling pH	Control pH (7.0)	Control pH	107.55	1.79
NaCl	0-3 %	2 %	308.72	5.15

Suggestions

The results of this work lead to the following suggestions:

1. Large scale production of 5-aminolevulinic acid using low cost by-product as culture medium such as tuna condensate as nitrogen source.
2. Applications of 5-aminolevulinic acid such as selective and biodegradable herbicide, insecticide and growth promoting factor.
3. Purification of 5-aminolevulinic acid from *Rhodobacter capsulatus* SN28.
4. Study on the mechanism of 5-aminolevulinic acid from *Rhodobacter capsulatus* SN28.
5. Development of the method on strain improvement for higher production of 5-aminolevulinic acid with low cost production.