REVIEW ARTICLE

5-aminolevulinic acid from photosynthetic bacteria and its applications

Amornrat Tangprasittipap[1] and Poonsuk Prasertsan[2]

Abstract

Tangprasittipap, A. and Prasertsan, P.
5-aminolevulinic acid from photosynthetic bacteria and its applications
Songklanakarin J. Sci. Technol., 2002, 24(4) : 717-725

This paper gives an overview on ALA production by photosynthetic bacteria concerning biosynthesis and regulation as well as its application as herbicide, insecticide and growth stimulator. Recent medical applications in the field of photodynamic therapy, cancer treatment, tumor diagnosis and other clinical uses are described.

Key words : 5-aminolevulinic acid, photosynthetic bacteria, ALA production, application

[1]Ph.D. Student in Biotechnology, [2]Ph.D. (Biotechnology), Assoc. Prof., Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112 Thailand.
Corresponding e-mail : ppoonsuk@ratree.psu.ac.th
Received, 28 February 2002 Accepted, 5 June 2002

5-Aminolevulinic acid (ALA) or 5-amino-4-oxo-pentanoic acid, is an aliphatic precursor of tetrapyrrole biosynthesis present in all living cells. ALA is the natural photodynamic compound effective as a biodegradable herbicide and insecticide harmless for crops, humans and animals (Sasikala *et al*., 1994) as well as having promotive effect on the growth and photosynthesis of crops and vegetables (Sasaki *et al*., 1993). Further applications of ALA are now in the area of medicine and pharmacy products (Levy, 1995).

Commercial ALA is produced by chemical synthesis, which involves many complex reactions and causes of high expenditure. Biological production of ALA by algae, anoxygenic photosynthetic bacteria and chemotrophic bacteria (Table 1) is an alternative approach as it is a less expensive method than chemical synthesis. Anoxygenic phototrophic bacteria (APB) can accumulate and excrete high concentration of ALA into the medium, hence it is suitable for commercial exploitation (Sasikala *et al*., 1994) and it now commercially produces from *Rhodobacter sphaeroides*.

This paper gives an overview on the biosynthesis and regulation of ALA by photosynthe-

Table 1. Production of ALA by different groups of microorganisms

Microorganisms	Carbon and nitrogen source	LA	ALA (μM)	References
Phototrophs				
Algae				
Agmemnellum quadruplicatum	Glutamate	+	0.225	Kipe-Nolt and Steven, 1980
Cyanidium caldarium	Glutamate	+	0.483	Jugenson *et al*., 1976
Bacteria, oxygenic phototrophic				
Anacystis nidulans	Glutamate	+	0.38	Anderson *et al*., 1983
Anabaena variabilis	Glutamate	+	0.019	Avisser *et al*., 1983
Bacteria, anoxygenic phototrophic				
Rhodobacter. sphaeroides	Succinate and glycine	+	0.75	Anderson *et al*., 1983
R. sphaeroides	Succinate and glycine	+	2-4	Sasaki *et al*., 1991
R. sphaeroides	Succinate and glycine	+	160.0	Ishii *et al*., 1990
R. sphaeroides	Swine waste (VFA)	+	4200	Sasaki *et al*., 1990
R. sphaeroides	Mandarin orange peel (modern synthetic waste water)	+	16000	Sasaki *et al*., 1993
R. sphaeroides	Sewage sludge	+	9300	Tanaka *et al*., 1983
Chlorobium limicola	Glutamate	+	3,950	Anderson *et al*., 1983
Chemotrophic bacteria				
Aerobes				
Pseudomonas riboflavina	L-alanine	+	0.2	Rhee *et al*., 1987
Propionicbacterium shermanii	Succinate and glycine	+	0.04	Menon and Shemin, 1967
Anaerobes				
Clostridium thermoaceticum	Glucose and L-lysine	+	155.0	Sjoji *et al*., 1989
Methanosarcina barkeri	Methanol, 2-oxoglutarate	+	0.4	Lin *et al*., 1989
Methanobacterium thermoautotrophicum	$H_2 + CO_2$	+	0.2	Lin *et al*., 1989

VFA : Volatile Fatty Acid ; LA : Levulinic acid ; + : Addition
Source : Sasikala *et al*., 1994

tic bacteria as well as its application as herbicide, insecticide and growth stimulator and in the medical fields.

Biosynthesis of ALA

The biosynthesis of ALA can be formed via two distinct metabolic pathways (Figure 1).

1. C_4 pathway (Shermin pathway)

Shermin pathway is observed in mammalian cell, yeast, fungi and very common among the purple non-sulfur photosynthetic group and a few chemotrophs (Sasaki *et al*., 1990). The key enzyme involved in the C_4 pathway of ALA formation is ALA synthetase (EC 2.3.1.37) catalyzing the condensation of succinyl-CoA and glycine. The ALA synthetase activity was highest from cells harvested at the logarithmic phase of growth (Sato *et al*., 1985).

The culture conditions have high influence on the synthesis of ALA synthetase and changing the culture condition from aerobic to micro-aerobic increased the ALA synthetase activity by 2-4 fold (Sandy *et al*., 1985). On the other hand, the enzyme synthesis in light was repressed by oxygen and the effect could be overcome upon the restoration of anaerobic condition (Viale *et al*., 1983).

2. C_5 pathway

The C_5 pathway is present in higher plants, algae and several bacteria, (Kajiwara *et al*., 1994), indicating the purple and green sulfur bacteria (Sasikala and Ramana, 1995). In C_5 pathway, ALA is formed from glutamate or the α-ketoglutarate via a path that does not involve the ALA synthetase reaction. The purification of the C_5 pathway enzyme indicates that glutamate is reduced to ALA in three steps (Sasikala *et al*., 1994).

a) Ligation of t-RNA to glutamate catalyzed by glutamyl-t-RNA synthetase
b) Reduction of glutamyl-t-RNA to generate glutamate-1-semialdehyde (GSA) catalyzed by glutamyl-t-RNA reductase (EC 6.1.1.17)
c) Transamination of GSA to generate ALA catalyzed by GSA aminotransferase (GSA-AT, EC 5.4.3.8)

Two catalytic mechanisms (Figure 2) have been proposed for the GSA conversion to ALA (Grimm *et al*., 1991). ALA is formed by accepting and releasing amino group at position 5 and 4 of GSA, respectively (Figure 2a) or two molecules of GSA oriented head to tail formation to amino-hemiacetal dimer, which converted into a double Schiff base and rearranged into the amino-hemiacetal (exchange amino groups) and subsequently dissociate into two molecules of ALA (Figure 2b) (Sasikala *et al*., 1994).

Regulation of 5-aminolevulinic acid production in photosynthetic bacteria

1. Carbon and nitrogen sources

Although glutamate is the carbon and nitrogen source for ALA production by many microorganisms, ALA production can also be produced with other carbon sources (Sasikala *et al*., 1994). Glucose had the advantage of being are inexpensive source for the industrial production of ALA. In the batch fermentation of mutant strain of *R. sphaeroides* CR606 accumulated ALA to level of 20 mM after 18 h with the production rate was 1.1 mMh^{-1} and the yield coefficient for ALA was 40% (mol/mol) of glucose (Nishikawa *et al*., 1999).

R. sphaeroides can utilize volatile fatty acid (VFA) such as acetic, propionic and butyric acid as carbon and energy sources (Sasaki *et al*., 1987). In addition, VFAs produced from the anaerobic digestion liquor of sewage sludge was reported to be the carbon source for production of ALA, up to 9.2 mM, by *R. sphaeroides* with repeated addition of the glycine and glutamic acid as the organic nitrogen source (Tanaka *et al*., 1994). The effluent of anaerobic mandarin orange peel (Tinpi) supplemented with glycine could be used to produce 5-aminolevulinic acid from *R. sphaeroides* (Sasaki *et al*., 1993).

2. Precursors

The addition of precursors (succinate and glycine) in the range of 20-60 mM into the digestion liquor medium resulted in the positive effect to ALA production of *R. sphaeroides* IFO

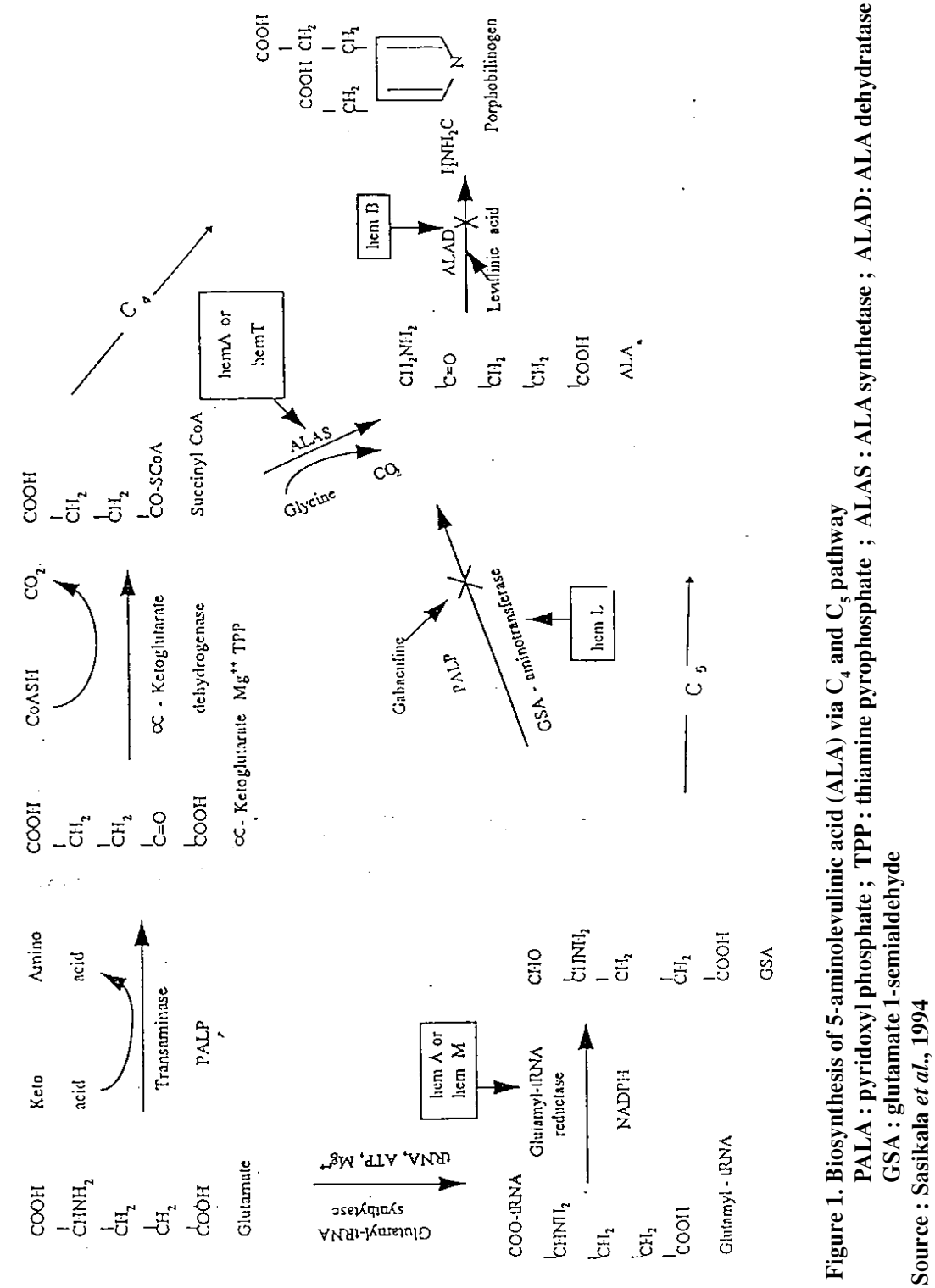

Figure 1. Biosynthesis of 5-aminolevulinic acid (ALA) via C_4 and C_5 pathway
PALA : pyridoxyl phosphate ; TPP : thiamine pyrophosphate ; ALAS : ALA synthetase ; ALAD: ALA dehydratase ; GSA : glutamate 1-semialdehyde
Source : Sasikala *et al.*, 1994

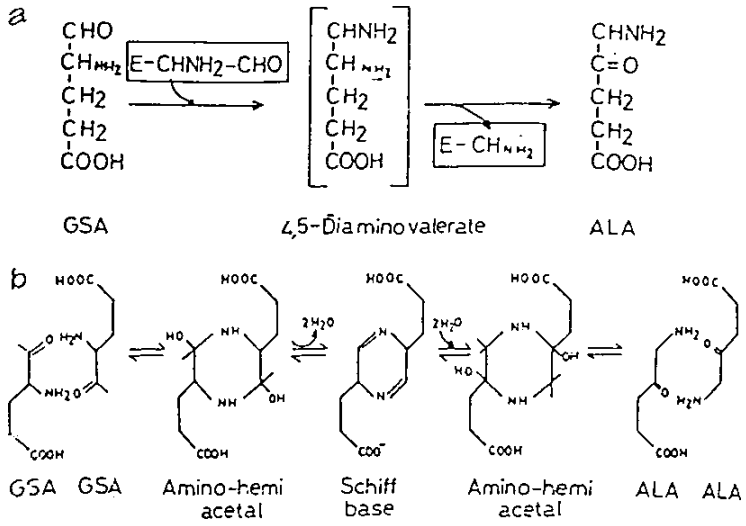

Figure 2. Mechanism of the conversion of glutamate 1-semialdehyde to ALA
Source : Grimm *et al.*, 1991

12203, but above 80 mM of precursors resulted in a negative effect on ALA formation. The supply of succinate is sufficient but the glycine supply might limit ALA formation in this culture system (Sasaki *et al*., 1990). ALA synthetase and ALA dehydratase activities in the cells are not influenced by the simultaneous addition of precursors compared with these activities in the control (no addition of precursor) but the growth is excessively suppressed by the addition of glycine (Sasaki *et al*., 1991).

The growth and ALA production of *Chlorella* sp. strain 4S is enhanced by glutamate addition, whereas the addition of succinate and glycine suppresses both (Sasaki *et al*., 1995). However, the addition of glutamate to the heterotropic medium culture of *Chlorella regularis* YA-603 does not enhance ALA production and Shermin pathway is suggested to contribute to ALA production of this strain (Ano *et al*., 1999).

3. Levulinic acid

Levulinic acid (LA), analog of ALA, is an inhibitor of ALA dehydratase which enhances extracellular ALA formation (Sasaki *et al*., 1987). In photoheterotrophic culture of *R. sphaeroides*, repeated addition of LA results in moderate cell growth suppression while extracellular ALA is not produced during the cultivation without the addition of LA. The amount of LA should be as small as possible since LA is expensive compared with glycine and 30 mM LA are recommend for use to save cost (Sasaki *et al*., 1990). At LA concentration over 50 mM, growth ceased completely and ALA was not excreted (Sasaki *et al*., 1987).

4. Metal ions

Metal ions particularly Fe^{2+} and Co^{2+} are important elements for regulating tetrapyrrole biosynthesis in *R. sphaeroides*. ALA synthetase is regulated by heme compound as feedback inhibition or repression under iron- sufficient conditions. Therefore, ALA production medium should contain neither cobalt nor iron to enhance ALA accumulation (Sasikala *et al*., 1994).

5. Light intensity

Light intensity is an important factor for enhancing ALA formation. Growth of photosynthetic bacteria is found to be independent of light intensity (1-5 klux), while the amount of ALA is reached the maximum value at 3 klux. High illumination (over 5 klux) was not effective for ALA production and low illumination (below 1 klux) produced quite a low growth rate and virtually no formation of ALA (Sasaki *et al*.,

1987).

6. Aeration

Oxygenation is one of the important factors affecting ALA synthetase activity in the loss of pigmentation due to the decrease in the ALA synthetase activator, cysteine trisulfide and glutathione trisulfide (Sandy *et al*., 1985). Changing the culture conditions from aerobic to microaerobic increased the activity of ALAS by 2-4 folds. The biosynthesis of ALA synthetase under light condition was repressed by oxygen and the effect could be overcome upon the restoration of anaerobic conditions (Viale *et al*., 1983).

7. pH

The effect of pH (6.0-8.0) of the VFAs culture medium is studied on ALA production by *R. sphaeroides*. At neutral pH (6.8 and 7.0) extracellular ALA production is up to 16 mM. Under controlled pH 6.8±1, intracellular ALA synthetase activity is significantly enhanced after adding LA, while ALA dehydratase is inhibited to low level. At higher pH (8.0) ALA synthetase activity was low and ALA dehydratase is relatively high (Sasaki *et al*., 1993). The inhibitory effect of LA for ALA dehydratase activity of *R. sphaeroides* is strongly dependent on the pH value of the GM medium. At pH 5.5, 5 mM LA inhibited 85% of ALA dehydratase activity (*in vitro*), while 100 mM LA decreased 45% of ALA dehydratase activity at pH 7.5 (Sasaki *et al*., 1997).

8. Others factors

Biotin was needed for ALA synthetase activity in the formation of ALA which is the intermediate of bacteriochlorophyll synthesis. Thiamine is the substrate of the coenzyme thiamine pyrophosphate which converts α-oxaglutarate to the C_4-intermediate of Shermin pathway (Lascelles, 1956).

Low molecular weight sulfur compounds of cysteine or glutamine, cysteine trisulfide (CySSSCy), glutathione trisulfide (GSSSG), glutathione and cysteine trisulfide (GSSSCy) and trisulfanedisulfonate ($S_5O_6^{2-}$) regulated the activity of ALA synthetase *in vivo*. Poly (sulfane) disulfonate ($-O_3S-S_n-SO_3^-$) and R-S*n*-R' (R and R' are organic or inorganic group) with $n > 3$ are exhibited as the activators of ALA synthetase. LA requires the presence of an exogenous thio, such as 2-mercaptoethanol or dithioerythritol to maintain catalytic activity while nitrite in the growth medium inhibited ALA production (Sasikala *et al*., 1994).

Application of 5-aminolevulinic acid

5-aminolevulinic acid converts molecular oxygen into singlet oxygen when excited by the absorption of light. ALA is potentially useful in agriculture as a herbicide and can be used as an antimicrobial drug. The important reason is that it is nontoxic to mammals, is readily biodegradable, and has no adverse effects on the environment (Tanaka *et al*., 1992). ALA can also be applied as photodynamic therapy for malignant skin tissue tumors.

1. Herbicide

ALA has photodynamic herbicide properties under appropriate treatment conditions. An immediate dark-incubation period after spraying ALA is an essential step to accumulate tetrapyrrole within the plants. ALA serves as a building block of tetrapyrrole accumulation, while a group of modulators, *O*-phenanthroline, ethyl nicotinate and 2,2'-depyridyl (DP), have affected to the pattern of tetrapyrrole accumulation and act in concert with ALA. DP is a cheap chemical then it is selected to mix with ALA to enhance the accumulation of tetrapyrrole. During the daylight period, the excess tetrapyrrole produces active oxygen (singlet oxygen) which oxidizes the unsaturated fatty acid on the cell surface (lipoprotein component), thus setting in motion a greatly damaging free-radical chain reaction. The cell membranes become leaky and this in turn results in a rapid and severe dehydration, bleaching and collapse of the leaf and/or hypocotyl tissue (Rebriz *et al*., 1984). Within 24 h the green plant tissue turns into a brownish desiccated mass of dead tissue (Rebriz *et al*., 1990). On the other hand, treated plants kept for the same period of time in darkness were unaffected.

The accumulation of tetrapyrrole in plant leaves causes very severe photodynamic

damage and the leave die within a few of hours while the cotyledons, stem and growing point remain unaffected. Dicotyledonous weeds such as redroot pigweed, purslane and lambquarter are highly susceptible to the tetrapyrrole induced photodynamic damage. Monocots such as core, wheat, oats and barley were not adversely affected by the spray (Rebriz *et al*., 1984). The death of plants depended on the ages of plant, ALA concentration, type of modulators, ratio of ALA and modulator, light intensity and kinds of plant treated (Kobayashi and Haque, 1971).

2. Insecticide

Rebriz and his co-worker (1988) developed a novel porphyrin insecticide consisting of modulator of porphyrin, 3.0 mM of ALA plus 30 mM of DP at pH 3.5. When this solution was sprayed on the larvae of *Trichoplusia ni* (Hubner insects) ALA induced the massive accumulation of protoporphyrin IX causing death in darkness via an unknown mechanism and in the light probably via singlet oxygen formation. Besides the advantages of ALA being nontoxic to nontarget organisms, such as other crops, animals and human, it is also difficult for insects to develop resistance against ALA.

3. Growth stimulator

Besides having herbicidal property, ALA is a very good growth simulator when used at low concentrations (Sasikala and Ramana, 1995). ALA has promotive effect on the growth and yield of several crops and vegetables, enhancing photosynthesis, stimulating fixation of CO_2 in light. The ALA also suppresses the respiration and the release of CO_2 under dark (Hotta *et al*., 1997). The appropriate applications of ALA showed 10-60% promotive effect over the control on radish, kidney beans, barley, potatoes, garlic, rice and corn (Sasikala *et al*., 1994). In addition, the culture medium from ALA production by *R. sphaeroides* could be directly used as a fertilizer having herbicide activity (Sasaki *et al*., 1990).

4. Photodynamic therapy

Photodynamic therapy (PDT) involves the use of photosensitizers (light-sensitive molecules) that are activated by light caused the formation of active forms of oxygen which is resulted in the killing of cell in which the photosensitizers are present, while sparing the normal surrounding tissue (Levy, 1995).

Kennedy *et al* (1990) proposed the use of tropically ALA based PDT for selected cutaneous disease. The cosmetic results of ALA-PDT treatment are very good and with a minimal effect on the normal skin (Sveanberg *et al*., 1994). Promising clinical results have been obtained in photosensitizing superficial skin tumors. In contrast, non-superficial and tumors of morpheaform histologic pattern have shown minor response rates only (Martin *et al*., 1995, Szeimies *et al*., 1994).

References

Anderson, T., Briseid, T., Nesbakken, T., Ormerod, J., Sirevaag, R. and Thorud, M. 1983. Mechanism of systhesis of 5-aminolevulinic acid in purple, green and blue-green bacteria. FEMS Microbiol. Lett. 19 : 303-306.

Ano, A., Watanabe, K., Tanake, T., Hotta, Y. and Nagai, S. 1999. Effect of glycine on 5-aminolevulinic acid biosynthesis in heterotrophic culture of *Chlorella regularis* YA-603. J. Ferment. Bioeng. 88 : 57-60.

Avissar, Y.J. 1980. Biosynthesis of 5-aminolevulinate from glutamate in *Anabaena variabilis*. Biochem. Biophys. Acta. 613 : 220-228.

Grimm, B., Bull, A. and Breu, V. 1991. Structural genes of glutamate 1-semialdehyde aminotransferase for porphyrin synthesis in cyanobacterium and *Escherichai coli*. Mol. Gen. Genet. 225 : 1-10.

Hotta, Y., Tanaka, T., Takaoka, H., Takeuchi, Y. and Konnai, M. 1997. New physiological effect of 5-aminolevulinic acid in plants : the increase of photosynthesis chlorophyll and plant growth. Biosci. Biotech. Biochem. 61 : 2025-2028.

Ishii, K., Hiraishi, A., Arai, T. and Kitamura, H. 1990. Light dependent porphyrin production by suspended and immobilized cells of *Rhodobacter sphaeroides*. J. Ferment. Bioeng. 60 : 26-32.

Kajiwara, M., Mizutani, M., Matauda, R., Hara, K. and Kojima, I. 1994. A new biosynthetic pathway of porphyrin form isopropanol. J. Ferment. Bioeng. 77 : 626-629.

Kennedy, J.C., Pottier, R.H. and Pross, D.C. 1990. Photodynamic therapy with endogenous protoporphyrin IX : basic principles and present clinical experience. J. Photochem. Photobiol. B. 6 : 143-148.

Kipe-Holt, J.A. and Stevens S.E. 1980. Biosynthesis of 5-aminolevulinic acid from glutamate in *Agmenellum quadruplicatum*. Plant. Physiol. 65 : 126-128.

Kobayashi, M. and Haque, M.Z. 1971. Contribution of nitrogen fixation and soil fertility by photosynthetic bacteria. Plant. Soil. 443-456.

Lascelles, J. 1956. The synthesis of porphyrins and bacteriochlorophyll by cell suspension of *Rhodopseudomonas sphaeriodes*. Biochem. J. 62 : 79-93.

Levy, J.G. 1995. Photodynamic therapy. TIBTECH. 13 : 14-17.

Lin, D., Nishio, H. and Nagai, S. 1989. Production of 5-aminolevulinic acid by methanogen. J. Ferment. Bioeng. 68 : 88-91.

Martin, A., Tope, W.D., Greveling, J.M., Starr, J.C., Fewkes, J.L., Hotte, T.J. Deutsch, T.F. and Andersson, R.R. 1995. Lack of selectivity of protoporphyrin IX fluorescence for basal cell carcinoma after topical application of 5-aminolevulinic acid: implication for photodynamic treatment. Arch. Dermatol. Res. 287 : 665-674.

Menon, I.A. and Shemin, D.J. 1967. Concurrent decrease of enzyme activities concerned with the synthesis of co-enzyme B12 and of propionic acid in Propionibacteria Arch. Biochem. Biophys. 121 : 304-310.

Nishikawa, S., Watanabe, K., Tanaka, T., Miyashr, N. and Murock, Y. 1999. Rhodobacter sphaeroides mutants which accumulate 5-aminolevulinic acid under aerobic and dark condition. J. Biosci. Bioeng. 87 : 798-804.

Rebriz, C.A., Montazer-Zouhour, A, Hoppen, H. and Wu, S.M. 1984. Photodynamic herbicides I : Concept and phenomenology. Enzyme. Microbiol. Technol. 6 : 390-401.

Rebriz, C.A., Jucick, J.A. and Rebriz, C.C. 1988. Porphyrin insecticide 1. Concept and phenomenology. Pestic. Biochem. Physist. 30 : 11-27.

Rebriz, C.A., Reddy, K.N., Navdihall, V.B. and Velo, J. 1990. Tetrapyrrole-dependent photodynamic herbicides. Photochem. Photobio. 52 : 1099-1117.

Rhee, H.I., Murata, K. and Kimura. A., 1987. Formulation of the herbicide 5-aminolevulinic aid form L-alanine and 4,5 dioxovalerate by *Pseudomonas riboflavin*. Agric. Biol. Chem. 51 : 1701-1702.

Sandy, J.D., Davies, R.C. and Neuberger, A. 1985. Control of 5-aminolevulinic synthetase activity in *Rhodopseudomonas sphaeroides*. A role of trisulphides. Biochem. J. 150 : 245-257.

Sasaki, K., Ikeda, S., Nishizawa, Y. and Hayashi, M. 1987. Production of 5-aminolevulinic acid by photosynthetic bacteria. J. Ferment. Techol. 65 : 511-515.

Sasaki, K., Tanaka, T., Nishizawa, Y. and Hayashi, Y. 1990. Production of herbicide, 5-aminolevulinic acid by *Rhodobacter sphaeroides* using the effluent of swine wastes from an anaerobic digester. Appl. Microbiol. Biotechnol. 32 : 727-731.

Sasaki, K., Tanaka, T., Nishizawa, Y. and Hayashi, M. 1991. Enhanced production of 5-aminolevulinic acid by repeated addition of levulinic acid and supplement of precursors in photoheterotrophic culture of *Rhodobacter sphaeroides*. J. Ferment. Bioeng. 71 : 403-406.

Sasaki, K., Tanaka, T., Nishizawa, Y. and Nagai, S. 1993. Effect of pH on the extracellular production of 5-aminolevulinic acid by *Rhodobacter sphaeroides* from volatile fatty acid. Biotechnol. Lett. 15 : 859-864.

Sasaki, K., Watanabe, M., Tanaka, T., Hotta, Y. and Nagai, S. 1995. 5-Aminolevulinic acid production by *Chlorella* sp. during heterotrophic cultivation in the dark. World. J. Microbial. Biotechnol. 11 : 361-362.

Sasaki, K., Watanabe, M. and Nishio, N. 1997. Inhibition of 5-aminolevulinic acid (ALA) dehydratase by undissociated levulinic acid during ALA extracellular formation by *Rhodobacter sphaeroides*. Biotech. Lett. 19 : 421-424.

Sasikala, Ch., Ramana, Ch.V. and Rao, P.R. 1994. 5-Aminolevulinic acid : A potential herbicide/ insecticide from microorganism. Biotechnol. Prog. 10 : 451-459.

Sasikala, Ch. and Ramana, Ch.V. 1995. Biotechnological potential of anoxygenic phtototrophic bacteria II. biopolyester, biopesticide, biofuel and biofertilizer. New York : Academic Press.

Sato, K. Ishida, K., Mutsushika, O. and Shimizu, S. 1985. Purification and some properties of δ-aminolevulinic acid synthetase from *Rhodobacter ruber* and *Rhodopseudomonas sphaeriodes*. Agri. Biol. Chem. 49 : 3415-3421.

Shoji, K.A., Nishio, N. and Nagai, S. 1989. Production of extracellular 5-aminolevulinic acid by *Clostridium thermoaceticum* grown in minimal medium. Biotech. Lett. 11 : 567-572.

Sveanberg, K., Andersson, T., Killander, D., Wang, I., Stenrum, U., Andersson-Engles, S., Berg, R., Johansson, J. and Svanberg, S. 1994. Photodynamic therapy of nonmalenoma malignant tumours of the skin using topical δ-aminolevulinic acid sensitization and laser irradiation. Br. J. Dermatol. 130 : 743-751.

Szeimies, R., Sassay, T. and Lanthaler, M. 1994. Penetration potency of topical applied δ-aminolevulinic acid for photodynamic therapy of basal cell carcinoma. Photochem. Photobiol. 59 : 73-76.

Tanaka. T., Takahashi, K., Hotta, T., Takeechi, Y. and Konnai, M. 1992. Promotive effect of 5-aminolevulinic acid on yield of several crops. Proc. 19th Annual Meeting of Plant Growth Regulator Society of America, San Francisco, USA.1992 : 237-241.

Tanaka, T., Watanabe, B., Watanabe, K., Nishikawa, S. and Hotta, Y. 1993. Cultivation method of bacteria and manufacture of tetrapyrrole by the method. Jpn. Kokai Tokko Koho JP 05224937.

Tanaka, T., Sasaki, K., Noparatanaraporn, N. and Nishio, N. 1994. Utilization of volatile fatty acids from the anaerobic digestion liquor of sewage sludge for 5-aminolevulinic acid production by photosynthetic bacteria. World. J. Microbiol. Biotechnol. 10 : 667-680.

Viale, A.A., Wider, E.A. and Battle, A.M.C. 1983. Some properties of the 5-aminolevulinate synthetase form Rhodopseudomonas pulustris. Rev. Argent. Microbiol. 15 : 233-238.