International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 12 (12), pp. 001-008, December, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Selection of photosynthetic bacteria producing 5-aminolevulinic acid from soil of organic saline paddy fields from the Northeast region of Thailand

Thanawan Kantha[1], Chaiyavat Chaiyasut[1]*, Duangporn Kantachote[2], Suchada Sukrong[3] and Amorntip Muangprom[4]

[1]Faculty of Pharmacy, Chiangmai University, Chiangmai, Thailand. [2]Faculty of Science, Prince of Songkla University, Songkhla, Thailand. [3]Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. [4]Laboratory of Plant Molecular Genetics, BIOTEC Central Research Unit, National Center for Genetic Engineering and Biotechnology, Thailand Science Park, Pathumthani, Thailand.

Accepted 02 June, 2018

Soil samples from organic saline paddy fields from 14 provinces of the northeast region of Thailand were collected and used for isolating photosynthetic bacteria (PB) prepared as a Soil and Straw Products (SSPs). PB from these SSPs were further grown in double strength G5 broth under microaerobic-light conditions before purification. A total of 130 isolates were then screened for growth in G5 broth under microaerobic-dark conditions for 24 h and 15 isolates were further selected by secondary screening in G5 broth under microaerobic-light conditions for 24 h. Four isolates (tk35, tk85, tk103 and tk123) were selected and all were identified as *Rhodopseudomonas palustris* based on their 16S rRNA gene. After incubation in SSP for 4 weeks with microaerobic-dark conditions, all SSPs had pH values in a range of 5.33 -7.17 and electrical conductivity (EC) values were between 3.02-12.93 dS/m. It was also found that the 5-aminolevulinic acid (ALA) content increased with time to achieve levels of 2.95, 2.94, 2.95 and 2.96 mM, from strains tk35, tk85, tk103 and tk123, respectively. The results indicate that SSP containing selected PB could produce ALA and this could be practically applied to organic saline paddy fields and increase growth and yields of rice.

Key words: Photosynthetic bacteria (PB), soil and straw product (SSP), 5-aminolevulinic acid (ALA), organic saline paddy field.

INTRODUCTION

Photosynthetic bacteria (PB) are widely distributed in nature especially in most submerged conditions such as paddy fields, ditches, soils in riverbeds and seashores, and sewage disposal plants (Kobayashi and Kobayashi, 2000). In addition, PB are common microorganisms in the natural environment and have been useful in the field of environmental protection such as in the treatment of sewage, household and restaurant wastewater (Nagadomi et al., 2000). Sasaki et al. (1998) reported that some PB such as Rhodospirillaceae can grow quickly under aerobic-dark conditions without utilizing light energy. In this situation, they utilize various type of organic matter as carbon and energy substrates. Some PB also produce relatively large amounts of physiologically active substances such as vitamin B12, ubiquinone and 5-aminolevulinic acid (ALA).

Koh and Song (2007) reported that two PB strains of *Rhodopseudomonas* sp. KL9 and BL6 produced an efficient growth enhancement of tomato seedlings under axenic conditions, together with the production of indole-3-acetic acid (IAA) and ALA. Solubilisation of insoluble phosphate may be responsible for the growth promotion of tomato seedlings. Lee et al. (2008) reported that application of *Rhodopseudomonas* sp. KL9 can enhance growth, fruit formation, yield and the quality of fruit in

*Corresponding author. E-mail: chaiyavat@gmail.com. Tel: +66-53-944340. Fax: +66-53-894163.

tomato plants in a greenhouse. PB have also been applied to high yielding cultures of rice. It was found that roots of control plants showed root rot, while those treated with PB showed good root development and growth, that was closely connected with an increase of yield (Kobayashi and Kobayashi, 2000) . Based on the above information and the ability of PB to fix N_2 (Raymond et al., 2004), PB can be considered to be one of natural biofertilizers.

The 5-aminolevulinic acid (ALA) is a key intermediate in the biosynthesis of tetrapyrroles, such as porphyrins, vitamin B12, chlorophyll (bacteriochlorophyll) and heme. ALA is a natural photodynamic compound effective as a biodegradable herbicide Sasikala et al., 1994) as well as having a promoting effect on the growth and photo-synthesis of crops and vegetables (Sasaki et al., 1993). In plants, the ALA concentration is strictly controlled at less than 50 nmol/g fresh weight (Stobart and Bukhari, 1984). Herbicidal activity has been reported to increase accumulation of several chlorophyll intermediates, such as protochlorophyllide, protoporphyrin IX and Mg-proto-porphyrin IX, when plants are treated with exogenous ALA at relatively high concentrations (5 – 40 mM).

It is assumed that the accumulated chlorophyll interme-diates act as photosensitizers for the formation of singlet oxygen, triggering photodynamic damage of ALA-treated plants (Chakraborty and Tripathy, 1992). However, low ALA concentrations, within the range of 0.06 - 0.60 mM, appear to promote rather than damage plant growth by increasing nitrate reductase activity, increasing fixation of CO_2 in the light, and suppressing the release of CO_2 in darkness (Hotta et al., 1997a). The effects of ALA on plant growth at low concentrations have been discovered to be physiologically different from those at high concentrations.

Rice is the most important food crop in the world. Almost half of the world's population depends on rice as their staple food. In Thailand, rice is a major culture and most of its production originates from irrigated fields in the northeast region. However, there are major environ-mental limitations on rice production in this region such as salinity and drought. In addition, recently, there has been an increased awareness of food safety worldwide, including Thailand, that has resulted in developing alternative ways to produce crops by 'organic agriculture'. The use of artificial fertilizers is not allowed in organic farming and thus biofertilizers like PB, that can produce ALA would be of interest for use in organic saline paddy fields. In agricultural systems, crop production is severely influenced by unfavourable environmental conditions causing great losses in productivity.

Several research reports have shown that ALA could be used in agricultural applications as a herbicide, insecticide and growth promoting factor including encouraging salt and cold temperature tolerance in plants (Sasaki et al., 1998; Watanabe et al., 2000). Tanaka et al. (1992) found that a low concentration of ALA application increased the chlorophyll content and accelerated the growth of plant tissue and rice seedling.

From the above benefits of PB and ALA, it was of interest to us to establish if PB can produce ALA, in organic saline paddy fields. Therefore, the aims of this study were to screen and select salt resistant PB strains able to produce ALA in soil and straw products (SSPs). In addition, some isolated PB strains with these properties were identified by 16S rRNA sequence analysis.

MATERIALS AND METHODS

Soil sample collection

Soil samples, from organic saline paddy fields from the northeast region of Thailand over 14 provinces included Sisaket, Amnatcharoen, Khonkaen, Nongkhai, Kalasin, Surin, Nakhonphanom, Udonthani, Mukdahan, Mahasarakham, Ubonratchathani, Roi et, Loei, and Nakhonratchasima were collected during the years 2007 - 2008.

Isolation of photosynthetic bacteria (PB) from Soil and Straw Products (SSPs)

This is the first report to show that photosynthetic bacteria (PB) producing ALA can be isolated from soil or sediment by using soil and straw products (SSP). In addition, this work introduces the concept that PB could be used by farmers as a biofertilizer and with straw may provide extra nutrients to improve soil fertility. As it is very difficult to directly isolate PB from soil or sediment, an additional enrichment step was included that involved growth in SSP under anaerobic-light conditions (Pfenning, 1989). We also used double strength G5 broth and this is a slight modification of the multiple tube fermentation technique because this technique allows the use of large sample sizes. With these modifications we had previously succeeded in isolating PB (Kantachote et al., 2005). In addition, 0.25% NaCl (4 dS/m), the average concentration of NaCl found in organic paddy fields in northeast region of Thailand, was added to the SSP/medium with the aim to isolate salt tolerant PB that produce ALA for use as an inoculum for organic saline paddy fields.

Each SSP for enrichment was prepared by mixing a bulk of 1 cm rice straw pieces, 1 g soil sample with 0.25% NaCl solution to cover 3/4 volume of a plastic bottle (600 ml). SSP bottles were incubated at room temperature under 3,000 lux light intensity. After 7 - 10 days incubation, 5 ml of the SSP was inoculated into 5 ml of double strength G5 broth followed by pouring 1 ml of sterile liquid paraffin onto the top of the growth medium held in a test tube. It was incubated at room temperature under a light intensity of 3,000 lux for about 7 days until the medium became colored that is reddish. After that, paraffin was removed. Colored precipitates were streaked to G5 agar in anaerobic-light conditions to purify single colonies.

Selection of photosynthetic bacteria

Primary and secondary screening were done under dark and light conditions with a small amount of O_2 because PB especially the purple nonsulfur photosynthetic bacteria are able to grow under various conditions that is anaerobic-light, microaerobic-light-conditions (Pfenning, 1989) or in the dark either aerobically or, microaerobically by using various organic compounds as carbon sources and electron donors (Akiba et al., 1983). It is well recognized that anaerobic-light conditions can promote ALA

formation by PB while oxygen represses ALA formation (Tanaka et al., 1991). This is due to the fact that ALA synthase has 2 forms (Form I and Form II) which are repressed under a high partial pressure of oxygen, but Form I can be induced by a reduction of the oxygen alone and the induction of Form II requires illumination (Tuboi et al. 1970).

Hence, based on the above information, experiments were designed to select PB as described. There are 4 reasons for using microaerobic-dark conditions to select for PB as a primary screening tool: The possibility of more ALA production (compared to aerobic -dark); inexpensive production; conditions at night time in the paddy fields sediment, and practical to produce by farmers. The first property of PB, is that they must grow well under microaerobic-dark conditions. Microaerobic-light conditions were designed for the secondary screening step for 2 reasons as follows: More ALA production (compared to microaerobic -dark) and following the conditions of the sediments in the paddy fields during the day time. The experiments as above were designed because isolated strains will be used as inoculants for promoting rice growth in saline paddy fields and also to explore an appropriate technology for farmers rather than scientists.

For primary screening**,** each isolate was incubated in G5 broth containing 0.25% NaCl. It was kept under microaerobic-dark conditions for 24 h. Bacterial growth was measured by a spectrophotometer at a wavelength of 660 nm. Sterile G5 broth containing 0.25% NaCl was also used as the blank. For secondary screening**,** 15 isolates with a high growth rate from the primary screening were streaked on G5 agar and kept for 24 h in the light. One loopful was then inoculated into G5 broth containing 0.25% NaCl. They were incubated for 24 h under microaerobic -light conditions with a 3,000 lux light intensity. Bacterial growth was measured spectrophotometrically at a wavelength of 660 nm. Sterile G5 broth containing 0.25% NaCl was also used as the blank.

Properties of SSPs containing photosynthetic bacteria

Starter cultures of strains (tk35, tk85, tk103 and tk123) were separately prepared by inoculating one loopful of each active culture into G5 broth and incubating under microaerobic-dark conditions for 48 h. The culture broth of each isolate was centrifuged at 8000 rpm (Sorvall RC 5C Plus) and washed twice with 0.85% NaCl. Cell pellets of each isolate were then suspended in 0.25% NaCl and the cell suspensions adjusted to obtain an OD_{660}nm of 1.0 for use as an inoculum. Sterile SSP was inoculated with a 10% inoculum size of each isolate and kept under microaerobic-dark conditions for 4 weeks with the aim to maintain live cells and/or a little proliferation for use in rice cultivation. In this experiment, SSPs were prepared as previously mentioned, except that SSPs were sterilized before inoculation. Samples were taken after weeks 1, 2, 3 and 4 to measure pH, electrical conductivity (EC) and ALA.

ALA was determined by a colorimetric method, at 553 nm, modified from the method of Mauzerall and Granick (1956). The main reason to monitor EC and pH values is that both parameters can affect plant and bacterial growth including their ALA production. It has long been known that EC estimates the total amount of dissolved ions in the water and EC value at more than 2 dS/m are toxic to rice (Department of Agriculture, 2005).

Identification of photosynthetic bacteria

For isolating genomic DNA, a pure culture of 4 PB isolates were grown overnight on G5 agar Genomic DNA was extracted with the method described by Hiney et al. (1992) with some modification. The PCR reaction was performed in a gradient thermal cycler (Eppendorf, Germany). The universal primers UFUL (5'-GCCTAACACATGCAAGTCGA-3') and URUL (5'-CGTATTACCGCGGCTGCTGG-3') were used for the amplification of the 16S rRNA gene fragment. The reaction mixture of 20 l consisted of 2 - 5 l of genomic DNA, 1 U of Taq DNA polymerase (BioLab), 1X PCR buffer pH 8.8 (10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl, 2 mM $MgSO_4$, 0.1% Triton X- 100) , 0.4 M dNTP and 0.4 M each of the two universal primers. Amplification was done by initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, the annealing temperature of the primers was 55°C for 30 s and extension at 72°C for 30 s. Final extension was at 72°C for 5 min. 10 l of the reaction mixture was then analyzed by gel electrophoresis using 1.5% agarose with ethidium bromide and the reaction product was visualized using a Gel doc/UV transilluminator.

100 ng of the 16S rDNA amplified PCR product was then used for sequencing with primer UFUL (5'-GCCTAACACATGCAAGTCGA-3') using the Automate DNA sequencer (3100-Avant Genetic Analyzer, ABI). A comparison of the 16S rRNA gene sequence of the 4 strains, with those in the NCBI website (http://www.ncbi.nlm.nih.gov/) used Nucleotide-nucleotide BLAST (blastn). All sequences of the four isolated PB were classified using the classifier tool and sequence match function in the Ribosomal Database Project II (RDP-II) release 10 (http://rdp.cme.msu.edu/) (Wang et al., 2007). A phylogenetic tree was constructed with tree builder tool in RDP-II. The type strain and reference sequences that gave the best match with the sequence from GenBank was also included in the phylogenetic tree. Bootstrapping values are indicated in the tree (Wang et al., 2007).

Statistical analysis

The analysis of pH, EC and ALA values were performed with Microsoft Excel 2007 (Window XP) for the mean and standard deviation of the three replicates. The statistical analysis was performed using their statistical difference by analysis of variance (ANOVA) (Ogbeibu, 2005). Mean comparisons were performed by Duncan's multiple range test. Analysis was carried out using SPSS 11.0 for windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Isolation and selection of photosynthetic bacteria from Soil and Straw Products (SSPs)

A total of 130 isolates of PB were made from SSPs containing soil samples from organic saline paddy fields collected from the northeast region of Thailand. According to the primary screening, the growth ability of all isolated PB under microaerobic-dark conditions when incubated for 24 h was classified into 3 groups. 104 isolates were classified into group 1 (poor growth, $OD_{660nm} < 0.1$) while 11 isolates were classified into group 2 (moderate growth, OD_{660nm}, 0.1 - 0.2) and 15 isolates were classified into group 3 (good growth, $OD_{660nm} > 0.2$). The 15 isolates of group 3 were further selected by secondary screening under microaerobic-light conditions when incubated 24 h (Figure 1). Four isolates; tk85, tk103, tk123 and tk35 showed the best growth with OD_{660nm} values of 0.30, 0.29, 0.27 and 0.27, respectively. Hence, these 4 isolates had the best potential for growth with both microaerobic-dark and light conditions and they were used as inoculants for producing SSPs for further

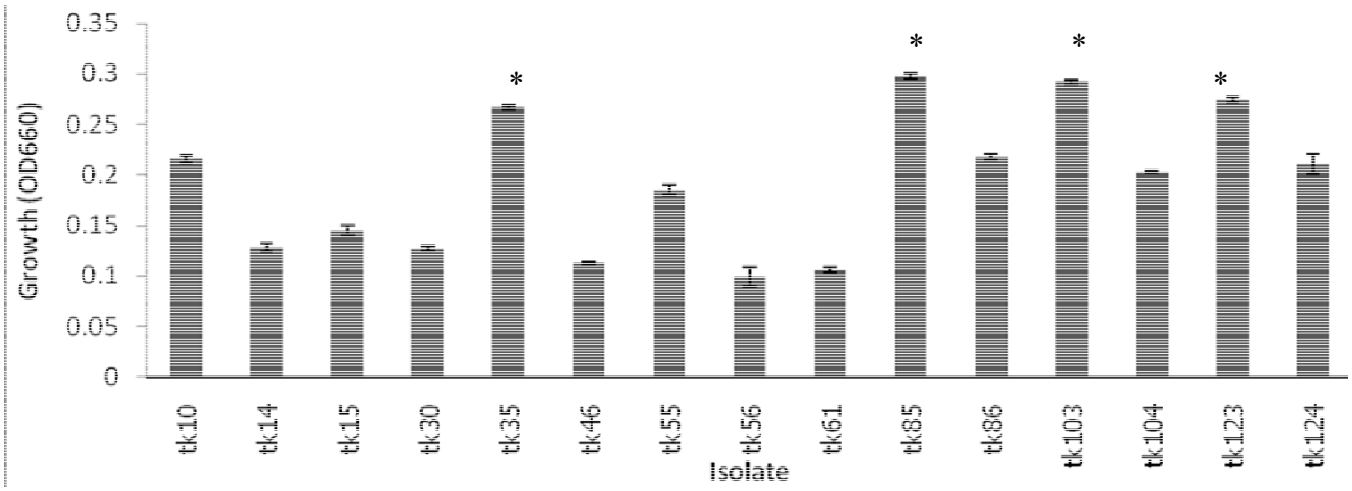

Figure 1. Growth of 15 isolates of photosynthetic bacteria in G5 broth plus 0.25%NaCl under microaerobic-light conditions after 24 h incubation. Each value is given as a mean ± standard deviation of triplicate determinations. Asterisk indicates the 4 selected isolates with better growth ($p < 0.05$) compared with other isolates.

Table 1. Change of pH and electrical conductivity (EC) values of sterile SSP containing individual strain of tk35, tk85, tk103 and tk123 under microaerobic-dark conditions and different incubation times. Values are given as mean ± standard deviation of triplicate determinations. The different superscripts in the same column denote a significant difference ($p < 0.05$).

Properties	Sterile SSP containing photosynthetic bacteria			
	tk35	tk85	tk103	tk123
pH value				
0 week	5.89 ± 0.05 ^{c}	5.66 ± 0.16 ^{d}	5.33 ± 0.30 ^{c}	5.70 ± 0.10 ^{d}
1 week	5.81 ± 0.08 ^{d}	6.85 ± 0.02 ^{b}	6.52 ± 0.06 ^{b}	6.20 ± 0.08 ^{c}
2 week	6.16 ± 0.01 ^{b}	6.99 ± 0.06 ^{b}	6.80 ± 0.03 ^{a}	6.58 ± 0.04 ^{b}
3 week	6.51 ± 0.03 ^{a}	6.21 ± 0.07 ^{c}	6.31 ± 0.01 ^{b}	6.90 ± 0.08 ^{a}
4 week	6.48 ± 0.02 ^{a}	7.17 ± 0.06 ^{a}	6.56 ± 0.04 ^{ab}	6.24 ± 0.08 ^{c}
EC (dS/m)				
0 week	3.02 ± 0.70 ^{e}	7.40 ± 0.78 ^{bc}	6.28 ± 0.09 ^{d}	3.17 ± 0.87 ^{c}
1 week	4.17 ± 0.45 ^{d}	7.98 ± 0.28 ^{b}	6.92 ± 0.14 ^{b}	6.13 ± 0.11 ^{b}
2 week	11.05 ± 0.29 ^{b}	7.05 ± 0.03 ^{c}	6.69 ± 0.04 ^{c}	7.78 ± 0.09 ^{a}
3 week	12.93 ± 0.68 ^{a}	7.38 ± 0.06 ^{bc}	6.74 ± 0.04 ^{c}	7.49 ± 0.14 ^{a}
4 week	7.68 ± 0.07 ^{c}	8.92 ± 0.03 ^{a}	7.80 ± 0.05 ^{a}	7.39 ± 0.05 ^{a}

testing.

Properties, including ALA production, of SSPs containing photosynthetic bacteria

Table 1 shows the pH and EC values of sterile SSPs containing individual isolates (tk35, tk85, tk103 and tk123). Each SSP had significantly different pH and EC values throughout their incubation time. The pH value of these SSPs ranged from 5.33 - 7.17. The pH values of SSPs at 4 weeks were higher than those at the beginning (t = 0). The EC value of SSPs was between 3.02 - 12.93 dS/m. The EC values at week 4 were higher than those at the beginning. However, EC values were variable at different incubation times. The quantities of ALA in these SSPs at different incubation time are shown in Figure 2. The ALA contents of these SSPs increased as the incubation time increased.

The amount of ALA in these SSPs at different incubation time were significantly different ($p < 0.05$), except at the 3 and 4 weeks incubation time of tk85 and tk103. The highest productions of ALA in each sterile SSP containing individual isolate of tk35, tk85, tk103 and tk123 at 4 weeks incubation were 2.95, 2.94, 2.95 and 2.96 mM, respectively.

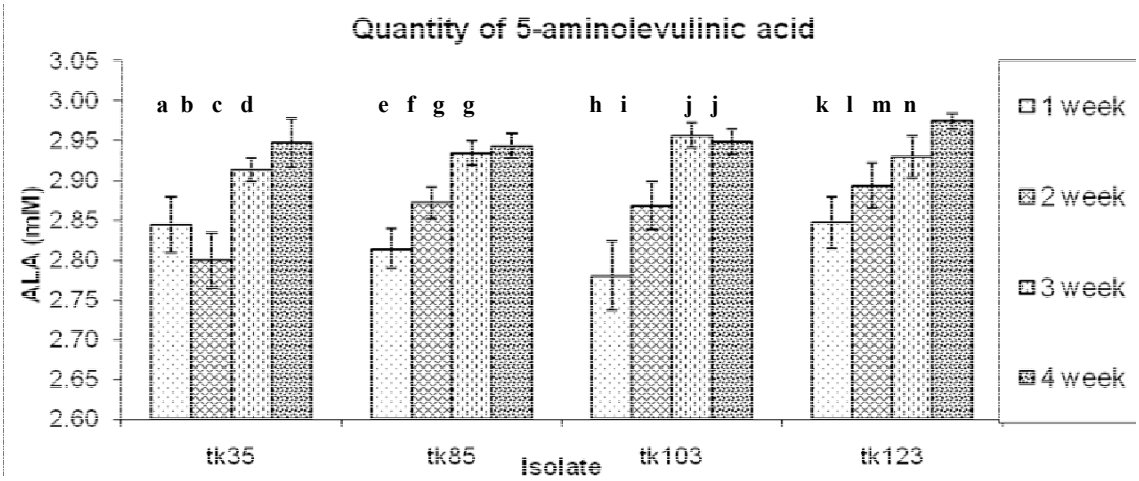

Figure 2. ALA contents of sterile SSP containing individual isolate of tk35, tk85, tk103 and tk123 under microaerobic-dark conditions after different incubation times Value is a mean ± standard deviation of triplicate determinations. Different lowercase letters indicate significant differences ($p<0.05$) among treatments.

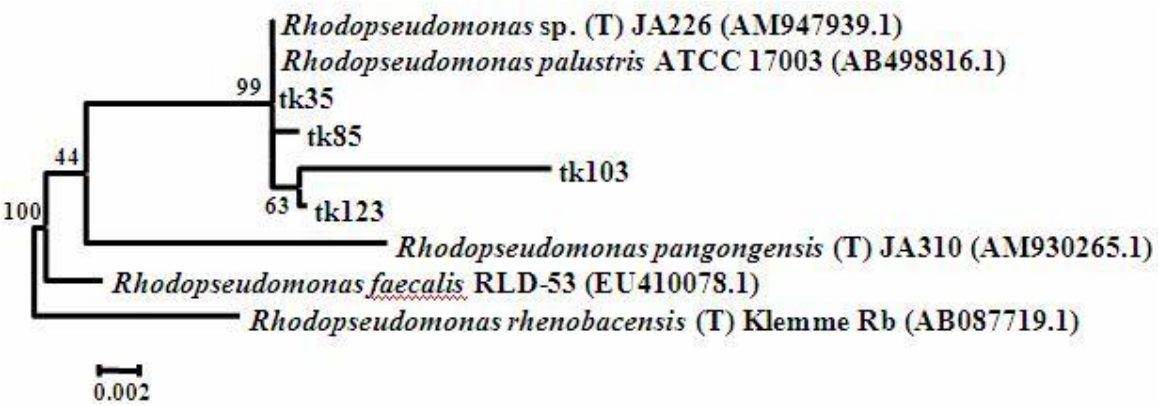

Figure 3. Phylogenetic tree of four isolated photosynthetic bacteria and related known bacterial species from RDP II. Accession number and bootstrapping value are indicated.

Identification of photosynthetic bacteria

All 4 isolates of PB (tk35, tk85, tk103 and tk123) were identified by the 16S rDNA sequence analysis. A significantly high degree of identity was found between the 4 isolates (99% similarity) with respect to a 500 bp of their 16S rRNA gene sequences. The consensus sequence determined for the *Rhodopseudomonas palustris* 16S rRNA gene was examined for sequence homology by using the NCBI, Gene Bank BLASTN function. For the sequence alignment, BLASTN was used to compare the 16S rRNA gene sequences and this produced a high homology of 99% with *R. palustris* (ATCC 17003) (Accession number AB498816.1). The sequence alignments with sequence derived from validated *R. palustris* species (derived from GenBank) indicated that the genus and species of the 4 tested isolates were all *R. palustris* (Figure 3).

DISCUSSION

Photosynthetic bacteria Isolations

This is the first report to use SSPs to isolate PB. As 130 isolates were obtained from 70 soil samples; however, not from 3 samples with very high amounts of salt (> 8 dS/m). Some samples gave more than one isolate but only distinctive PB colonies were selected for further studies. Based on our experience, it is not easy to directly isolate PB from soil as soil is a habitat enriched for heterotrophic microorganisms hence the need to carefully choose a medium specific for PB. Based on our results, SSP was a suitable medium to select PB from soil habitat. In our experiment, 130 isolates of PB were isolated from soil samples of organic saline paddy fields**.** However based on a primary screening by growth in G5 broth plus 0.25%NaCl under microaerobic-dark condition,

only 15 isolates that formed a group 3 with good growth were selected for secondary screening. Results indicated that they could resist salt and grew well under microaerobic-dark conditions that will be used as the incubating conditions in one step of a scale up process due to its cheap cost and possibly high ALA production.
From the secondary screening, 4 isolates; tk85, tk103, tk123 and tk35 had the best potential for growth with microaerobic- dark conditions. Therefore, they were identified using the 16S rRNA gene and all of them belonged to *R. palustris*. The results also indicated that each strain of *R. palustris* isolated from the soil of different organic saline paddy fields, in different areas, grew and survived in a salty medium (G5 + 0.25% NaCl) with both microaerobic-dark and light conditions. Hence, the 4 isolates were suitable for use in paddy fields because they can be cultured and grown with both microaerobic-light and dark conditions and these conditions are normally found in rice cultivation during day and night times, respectively.

Pfenning (1989) indicated that in nature, PB can be found in habitats such as fresh water, sea water, sulfur containing hot water springs and clay under anaerobic-light conditions. However, the genus *Rhodopseudomonas*, a group of purple non- sulfur bacteria, can grow in various metabolic conditions such as aerobic conditions, anaerobic-dark and anaerobic-light conditions (Kim et al., 2010). The four strains isolated from different areas; however, were the same species. It can be explained that methods of isolation and selection (adding 0.25% NaCl and microaerobic-dark/light conditions) would select such strains of *R. palustris* due to their physiological abilities being able to match the conditions used. In addition, their original paddy field habitats may allow these organisms to be the dominant species among the PB strains. Our results are supported by the work of Saikeur et al. (2009) who reported that *R. palustris* KG31 with an ability to produce ALA was isolated from soil collected from a paddy field in Nakhon Si thammarat province, Thailand.

Characteristics of PB in SSP

Four isolates were used as inoculants to produce SSPs by incubating under microaerobic-light conditions for 4 weeks. SSPs containing PB will be tested for use as a biofertilizer or growth promoter in a rice paddy field. Therefore, the activities of the SSPs must facilitate the growth of plants. The pH value is an important parameter to consider because it affects the availability of micro-nutrients. At a high pH, some nutrients that are essential for good plant growth become unavailable and the plants will start developing deficiency symptoms. The EC value can be used as an indicator of the presence of macronutrients in the growing SSPs (Lersel, 2000).

According to the physicochemical properties of SSPs in this experiment, the pH value of all incubated products ranged from 5.33 - 7.17. After 4 weeks incubation, there was a tendency of the pH values to decrease in the SSP of tk35 and tk 123 but with the SSP of tk85 and tk103, the pH had a tendency to increase. Moreover, the pH values of all SSPs were significantly different at different incubation times. However, the pH ranges of our products are appropriate for plants because they were in the range that is suitable for plants. It is well recognized that changes of pH occur due to metabolic activity of cells and thus the change of pH provides evidence that the bacterial cells are still active. The increase of pH in SSPs provided a suitable condition for *R. palustris* to produce more ALA as a neutral pH is optimal for producing ALA (Choi et al., 2004).

The EC value of each SSP was a measurement of the soluble materials such as minerals or nutrients. This will relate to the ability of the material to conduct electrical current through it (Gorby et al., 2006). EC values of all SSPs ranged from 3.02 - 12.93 dS/m and increasing along with the time of incubation. The metabolic activity of cells produced metabolites such as ALA and released it to the environment so that ALA increased in SSPs following the incubation time. The standard for the EC value is specified at not more than 10 dS/m in Thai organic fertilizer standards (Department of Agriculture, 2005). Hence, SSPs must be diluted prior to use as a plant promoter and this make SSPs inexpensive to use.

Role of ALA in promoting plant growth

ALA promotes the growth and yield of several crops and vegetables. ALA at low concentrations elicited a 10 – 60% promoting effect on radish, kidney beans, barley, rice, potatoes and garlic (Hotta et al., 1997b). In this study, it was found that over the 4 weeks of incubation, SSPs produced from pure cultures of PB produced ALA with the highest content of 2.9 mM. Zhang et al. (2002) reported that *Rhodopseudomonas* spp. are able to produce ALA and grow anaerobically in the light or aerobically in the dark using many carbon sources and electron donors. The reason why the ALA contents increased as the incubation time increased may indicate that all selected strains of *R. palustris* could grow well in SSPs and lead to an increased ALA content.

However, application of ALA to agriculture depends on the timing of the application, concentrations and type of plants. In the present study, the highest ALA content is sufficient to use in paddy fields and will need to be diluted to meet an appropriate level for stimulating plant growth. Hotta et al. (1997b) reported that when 0.18 and 0.6 mM of ALA were sprayed during the pre- flowering stage and the flowering stage, the grain yield of barley increased by 41 and 22%, respectively. These results indicated that ALA treatment might promote the ripening of the grains by increasing the supply of photosynthetic enzyme. Kobayashi et al. (1966) reported that PB, that produce ALA could provide favorable effects to the plants,

including an increase in the number of grains per rice plant. Therefore, any of the SSPs prepared in this study could be chosen to promote plant growth.

It will be necessary to further investigate the most appropriate times to apply ALA to plant cultures depending on whether seedling growth or yield enhancement is required (Hotta et al., 1997b). Gossett et al. (1994) reported that ALA application to plants protected them from cell damage because the increase of photosynthate induced by ALA could also lead to an increase of antioxidant enzymes. Zhang et al. (2006) reported that ALA at low concentrations of 0.3 – 3 mg/l promoted development and growth of potato microtubers *in vitro* and enhanced protective functions against oxidative stresses, but ALA at 30 mg/l and higher concentrations may induce oxidative damage.

Thus, it is important to clarify optimal application rates and timing of ALA for growth promotion of crops. Optimal rates may be slightly higher for crops growing under stressful conditions and the safety margin of application rates of ALA would be rather wide because ALA is rapidly metabolized in the environment. Based on the results, SSP was a suitable medium to select for PB as previously mentioned and also the suitable medium for maintenance culture. As over 4 weeks incubation time (testing time), it still retained a physiologically active concentration of ALA. Therefore, SSP has the potential for use as a biofertilizer in paddy fields. However, no enumeration of PB had been done in this study. Unlike, the current work counting of PB has been done. Further investigations, including use of bulked up SSP containing PB for testing under greenhouse and field conditions, are needed to clarify the role of ALA in promoting rice growth that will have beneficial effects on rice growth under salt stress conditions.

ACKNOWLEDGEMENTS

This study was supported by the Faculty of Pharmacy, Chiang Mai University, a grant from Thailand Graduate Institute of Science and Technology (TGIST), National Science and Technology Development Agency (NSTDA) and in part supported by Graduate School, Chiang Mai University. This work was carried out at Department of Microbiology, Faculty of Science, Prince of Songkla University as a collaborative research.

REFERENCES

Akiba T, Usami R, Horikoshi K (1983). *Rhodopseudomonas vutila*, a new species of nonsulfur purple photosynthetic bacteria. Int. J. Syst. Bacteriol., pp. 551-556.

Chakraborty N, Tripathy BC (1992). Involvement of singlet oxygen in 5-aminolevulinic acid induced photodynamic damage of cucumber (*Cucumis sativus* L.) chloroplasts. Plant Physiol., 98: 7-11.

Choi C, Hong JW, Rhee KH, Sung HC (2004). Cloning, expression, and characterization of 5-aminolevulinic acid synthase from *Rhodopseudomonas palustris* KUGB306. FEMS Microbiol. Lett., 236: 175-181.

Department of Agriculture (2005). Notification of the Department of Agriculture on Thai standards of organic fertilizer B. E.

Gorby YA, Yanina S, McLean JS, Rosso KM, Moyles D, Dohnalkova A, Beveridge TJ, Chang IS, Kim BH, Kim KS, Culley DE, Reed SB, Romine MF, Saffarini DA, Hill EA, Shi L, Elias DA, Kennedy DW, Pinchuk G, Watanabe K, Ishii S, Logan B, Nealson KH, Fredrickson JK (2006). Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. PNAS, 103: 11358-11363.

Gossett DR, Millhollon EP, Lucas MC, Banks SW, Marney MM (1994). The effects of NaCl on antioxidant enzyme activities in callus tissue of salt-tolerant and salt-sensitive cotton cultivars (*Gossypium hirsutum* L.). Plant Cell Rep., 13: 498-503.

Hiney M, Dawson MT, Heery DM, Smith PR, Gannon F, Powell R (1992). DNA Probe for *Aeromonas salmonicida*. Appl. Environ. Microbiol., 58: 1039-1042.

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

Hotta Y, Tanaka T, Takaoka H, Takeuchi Y, Konnai M (1997b). Promotive effects of 5-aminolevulinic acid on the yield of several crops. Plant Growth Regul., 22: 109-114.

Kantachote D, Torpee S, Umsakul K (2005). The potential use of anoxygenic phototrophic bacteria for treating latex rubber sheet wastewater. Electron Biotechnol., 8: 314-323.

Kim MS, Oh YK, Lee JK, Kim EJ (2010). Production of recombinant photosynthetic bacteria which produces molecular hydrogen in a light independent manner and hydrogen evolution method using above strain. United States Patent Application Publication: US2010/0003734.

Kobayashi M, Katayama T, Okuda A (1966). Seasonal changes of microbial counts in paddy field. J Sci Manure, Japan, 37: 441–446.

Kobayashi M, Kobayashi M (2000). Waste remediation and treatment using anoxygenic phototrophic bacteria. In: Blankenship RE, Madigan MT and Bauer CE, (eds): Anoxygenic Photosynthetic Bacteria, pp. 1269-1282.

Koh RH, Song HG (2007). Effects of application of *Rhodopseudomonas* sp. on seed germination and growth of tomato under axenic conditions. J. Microbiol. Biotechnol., 17: 1805-1810.

Lee KH, Koh RH, Song HG (2008). Enhancement of growth and yield of tomato by *Rhodopseudomonas* sp. under greenhouse conditions. The J. Microbiol., 46: 641-646.

Lersel van MW (2000). EC and pH: What is it? Southeastern Floriculture, 10(5): 11-14.

Mauzerall D, Granick S (1956). The occurrence and determination of -aminolevulinic acid and porphobilinogen in urine. J. Biol. Chem., 219: 435-46.

Nagadomi H, Takahasi T, Sasaki K, Yang HC (2000). Simultaneous removal of chemical oxygen demand and nitrate in aerobic treatment of sewage wastewater using an immobilized photosynthetic bacterium of porous ceramic plates. World J. Microbiol. Biotechnol., 16: 57-62.

Ogbeibu AE (2005). Biostatistics: A practical approach to research and data handling. Mindex Publishing Co. Ltd. Benin City. p. 264.

Pfenning N (1989). Ecology of phototrophic purple and green sulphur bacteria. In: Schlegel HG and Bowien B, (eds) Autotrophic Bacteria. Science Tech Publishers. pp. 97-117.

Raymond J, Siefert JL, Staples CR, Blankenship RE (2004). The natural history of nitrogen fixation. Mole. Biol. Evolution, 21: 541-555.

Saikeur A, Choorit W, Prasertsan P, Kantachote D, Sasaki K (2009). Influence of precursors and inhibitor on the production of extracellular 5-aminolevulinic acid and biomass by Rhodopseudomonas palustris KG31. Biosci. Biotechnol. Biochem., 73: 987-992.

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

Sasaki K, Tanaka T, Nagai S (1998). Use of photosynthetic bacteria for the production of SCP and chemicals from organic wastes. In: Bioconversion of waste materials to industrial products, second edition. Martin AM, (eds): Blackie Academic and professional, pp. 247-291.

Sasaki K, Watanabe M, Tanaka T,Tanaka T (2002). Biosynthesis, biotechnological production and applications of 5-aminolevulinic acid. Appl. Microbiol. Biotechnol., 58: 23-29.

Sasikala Ch, Ramana ChV, Rao PR (1994). 5-aminolevulinic acid: A potential herbicide/ insecticide from microorganisms. Biotechnol. Prog., 10: 451-459.

Stobart AK, Bukhari IA (1984). Regulation of -aminolevulinic acid synthesis and protochlorophyllide regeneration in the leaves of dark-grown barley (*Hordeum vulgare*) seedlings. Biochem. J., 222: 419-426.

Tanaka T, Watanabe k, Hotta Y, Lin D, Sasaki K, Nagai S (1991). Formation of 5-aminolevulinic acid under aerobic/dark condition by a mutant of *Rhodobacter sphaeroides.* Biotecnol. Lett., 13: 589-594.

Tanaka T, Takahashi K, Hotta T, Takeuchi Y, Konnai M (1992). Promotive effects of 5-aminolevulinic acid on yield of several crops. In Proceedings of the 19th annual meeting of plant growth regulator Society of America, San Francisco. Plant Growth Regulator Society of America, Washington DC: pp. 237-241.

Tuboi S, Kim H, Kikuchi G (1970). Occurrence and properties of two types of 6-aminolevulinate synthetase in *Rhodopseudomonas sphaeroides*. Archives Biochem. Biophy., 138: 147-154.

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007). Naïve bayesian classifier for rapid assignment to rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol., 73: 5261-5267.

Watanabe K, Tanaka T, Hotta Y, Kuramochi H, Takeuchi Y (2000). Improving salt tolerance of cotton seedling with 5-aminolevulinic acid. Plant Growth Regul., 32: 99-103.

Zhang D, Yang H, Huang Z, Zhang W, Liu SJ (2002). *Rhodopseudomonas faecalis* sp. nov., a phototrophic bacterium isolated from an anaerobic reactor that digests chicken faeces. Int. J. Syst. Evol. Microbiol., 52: 2055-2060.

Zhang ZJ, Li HZ, Zhou WJ, Takeuchi Y, Yoneyama K (2006). Effect of 5-aminolevulinic acid on development and salt tolerance of potato (*Solanum tuberosum* L.) microtubers *in vitro*. Plant Growth Regul., 49: 27-34.