Appendix

1. Viable Plate Count of the phototrophic bacteria

Example Calculations

The concentration of bacterial cells in the original culture is calculated from the dilution plating onto GM medium. The total dilution is calculated, its inverse is taken and then multiplied by the number of colonies on the plate:

55 colonies were observed when 0.1 ml of the 10^{-7} dilution was plated. The total dilution is: $1x\ 10^{-7}$ x 0.1 ml plated = 1×10^{-8}. The inverse of this is 1×10^{8}.

Colony forming units per ml (CFU/ml) are calculated by multiplying the dilution factor by the number of colonies: $55 \times (1 \times 10^{8}) = 55 \times 10^{8}$ or 5.5×10^{9} CFU/ml.

2. Dry cell weight of the phototrophic bacteria (Noparatnaraporn *et al.,* 1986)

Biomass content was determined gravimetry. Culture samples (10 ml) were centrifuged (10,000 *g*, 15 min, 4 °C), the supernatant was refrigerrated for further analysis, and the cell pellet was washed in deionized water, recovered (10,000 *g*, 15 min, 4 °C), dried to constant weight (90 °C, 24 h), cooled in desiccator, and weighed.

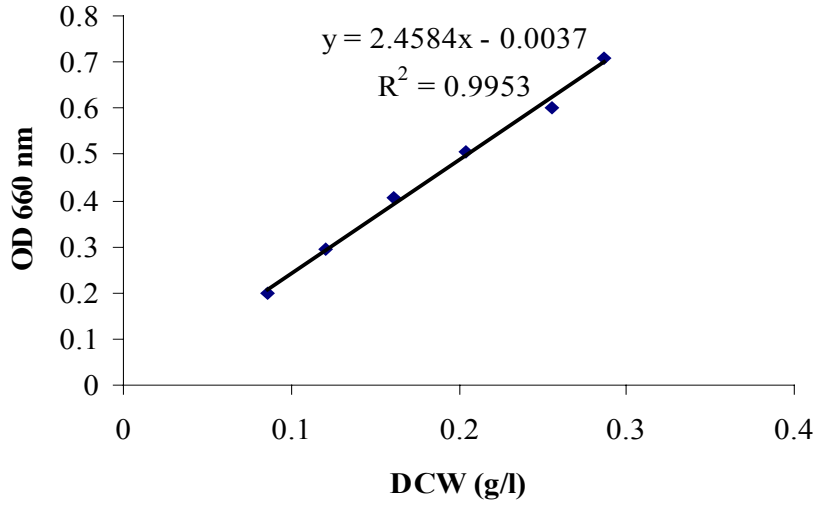

Fig 23. Standard curve of dry cell weight from strain SN28 cultivated in glutamate-glucose + 3 % NaCl under aerobic-dark condition at 37°C

2.Standard curve for extracellular ALA

Reagents

1. Acetate buffer 1 M pH 7.4
2. Acetylacetone
3. Modified Ehrlich's reagent. Prepared by adding 1 g of ρ-dimethyl-aminobenzaldehyde to 30 ml of glacial acetic acid, adding 8 ml of 70% perchloric acid and diluting to 50 ml with glacial acetic acid. (Must be prepared fresh daily)

Procedure

To a test tube was added 1 ml of standard ALA and 2 ml of acetate buffer. 0.05 ml of acetylacetone was added to each tube. ALA and acetylacetone condensed to form a pyrrole compound, 2-methyl-3-acetyl-4-(3-propionic acid) pyrrole, in the acetate buffer at 100 C. after heating for 15 min, the samples were quickly cooled on ice. Then 3.5 ml of Ehrlich reagent was added to each tubes. After 15 min, the optical density at 553 nm is determined.

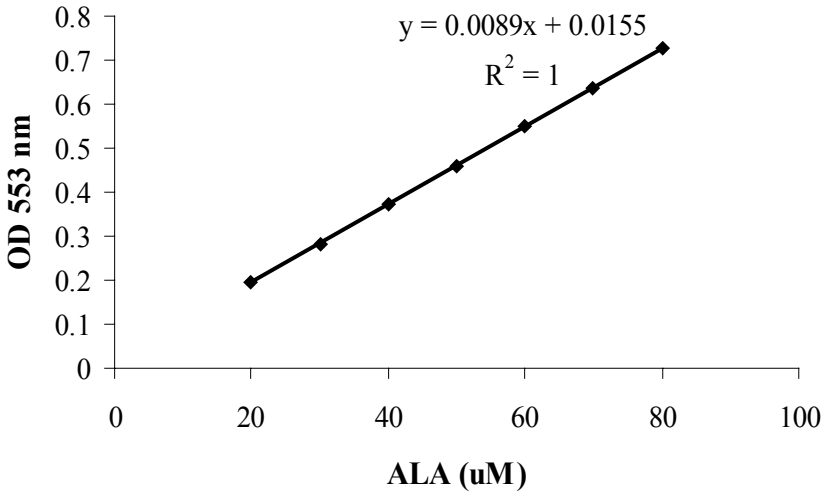

Fig 24 Standard curve of ALA

3.Standard curve for intracellular ALA

Reagents

1. Acetylacetone
2. Acetate buffer 1 M pH 7.4
3. 95% Ethanol
4. Sodium chloride
5. Formalin
6. Glacial acetic acid
7. Methanol (HPLC grade)
8. Water (HPLC grade)

Procedure

10 µl of Standard ALA (commercial ALA) in glass tube was added with 3.5 ml of a mixture acetylacetone, ethanol and water (15 : 10 : 75 ; by vol) containing 4 g of sodium chloride per liter and then with 450 µl of aqueous formalin (85 ml of formalin per liter). Heat the solution in boiling water for 30 min and then cooled in a water bath. The HPLC system (pre-column) with a fluorescence detector (Shimadzu, LC-10A, Osaka, Japan) was used by applying 20 µl solution to an injector sample loop. The column used was hyprsil ODS-3 (5 µM, 250 x 4.6 mm i.d., GL Science Inc. Tokyo, Japan) kept at 40 C. Finally the elution was performed with an aqueous solution containing methanol (HPLC grade; Merck) and 2.5% acetic acid (60:40 v/v) at a flow rate of 0.6 ml/min by a constant flow pump. Spectrofluorometer was used to monitor the fluorescence intensity of the eluate at 473 nm (excitation wavelength 363 nm). The correlation of peak area and standard ALA concentration were plotted as standard curve of intracellular ALA.

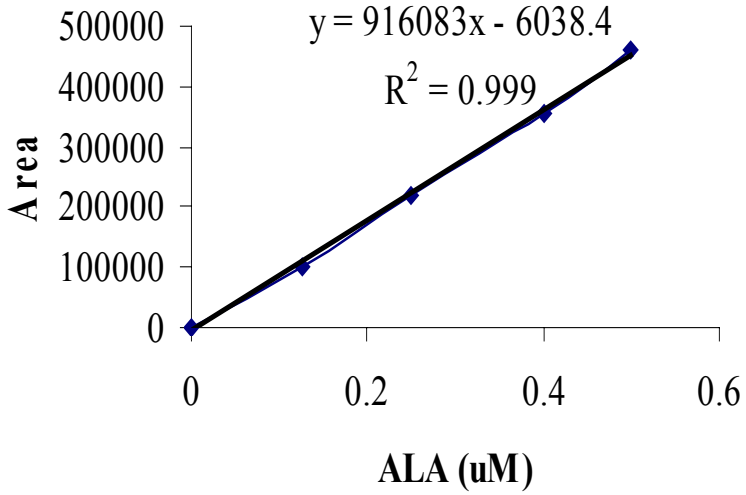

Fig25. Standard curve for Intracellular ALA

Publication

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