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Isolation, identification and growth conditions of photosynthetic bacteria found in seafood processing wastewater

P. Prasertsan,* W. Choorit and S. Suwanno

Four photosynthetic bacteria, isolated from 14 samples taken from seafood processing plants, were identified as species of *Rhodocyclus gelatinosus*, belonging to the purple, non-sulphur bacteria of the family *Rhodospirillaceae*. Cultivation in synthetic medium under four different conditions indicated that all four strains gave maximum carotenoid and bacteriochlorophyll synthesis under anaerobic conditions in the light, with values of 11 to 12.6 and 102 to 108 mg/g dry cell wt, respectively. These values are 87% higher than the pigment content obtained from aerobic cultivation, although the cell biomass of all strains (1.7 to 2.3 g/l) was 22 to 38% higher under aerobic conditions. Protein content was always between 32 and 43%. The specific growth rates of all isolates in aerobic cultivation (0.04 to 0.06 h^{-1}) were twice those in anaerobic conditions in the light. No growth occurred in anaerobic conditions in the dark.

Key words: Growth conditions, isolation, photosynthetic bacteria, *Rhodocyclus*, wastewater.

In nature, under anaerobic conditions in the light, photosynthetic bacteria can be found in habitats such as fresh water, sea water, sulphur-containing hot water springs, clay (Pfennig 1967; Imhoff 1988) and also in seafood-processing wastewater (Prasertsan & Choorit 1988). Photosynthetic bacteria are characterized by their ability to assimilate CO_2 and use light as their energy source (Pfennig & Truper 1974).

Photosynthetic bacteria are reported to contain 40 to 69% (w/w) protein, 0.09 to 0.80 mg carotenoid/g dry cell wt, 30 to 79 mg vitamin B_{12}/kg dry cell wt and an essential amino acid composition comparable with egg, algae and soybean (Kobayashi & Kurata 1978; Vrati 1984). In consequence, these bacteria have a potential use as a protein source in animal feed.

Materials and Methods

Cultivation Medium

The synthetic medium (G5) used for the enrichment and isolation of photosynthetic bacteria contained (g/l): peptone, 5.0; yeast extract, 5.0; L-glutamic acid, 4.0; KH_2PO_4, 0.12; and K_2HPO_4, 0.18 (Kohlmiler & Gest 1951). The initial pH of the medium was adjusted to pH 7.0 using 5 M NaOH. For the maintenance of the cultures, G5 agar (15 g agar/l G5 liquid medium) was used.

The basal medium, used for the identification of the isolates, was G5 supplemented with (mg/l): thiamine, 1.0; *p*-aminobenzoic acid, 1.0; nicotinic acid, 1.0; and biotin, 0.015.

Analytical Methods

The growth of the culture was measured turbidometrically at 680 nm and converted to cell dry wt using a calibration curve. Cell pigments (carotenoids and bacteriochlorophyll) were determined following the methods described by Hirayama (1968). Chemical oxygen demand (COD) was analysed according to the American Public Health Association (1985) and protein was measured using the Kjeldahl method (Association of Official Analytical Chemists 1984).

Isolation and Identification

Samples were collected from wastewater treatment systems of three seafood-processing factories in Songkhla, Thailand. The growth of the photosynthetic bacteria in the samples was enhanced by incubation under anaerobic conditions and 800 Lux ('anaerobic-light') at room temperature (approx 30°C) for 4 days, followed by enrichment in G5 medium (Kohlmiler & Gest 1951). A loopful of pinkish culture broth was streaked onto G5 agar without vitamins. After 7 days' incubation, each colony was picked up and streaked onto new G5 agar plates. This procedure was repeated until pure cultures were obtained.

P. Prasertsan, W. Choorit and S. Suwanno are with the Department of Agro-Industry, Faculty of Natural Resources, Prince of Songkla University, Hatyai 90110, Thailand; fax: + 66 74212823. *Corresponding author.

The isolates were identified according to the procedures described by Watanabe *et al.* (1981).

Effects of Growth Conditions

The isolates were cultivated in G5 medium under four different conditions: 'aerobic-dark', 'aerobic-light', 'anaerobic-light' and 'anaerobic-dark'. The aerobic condition was provided by placing the cultivation flasks on a shaker at a speed of 200 rev/min. Anaerobic conditions were produced by static cultivation and covering the medium surface with a 1-cm thick layer of liquid paraffin. A fluorescent lamp was used as the light source. Darkness was created by wrapping the cultivation flask with aluminium foil.

Results and Discussion

Four strains (T6, R4, R5, R7) of photosynthetic bacteria were isolated from the seafood-processing wastewaters. Since the isolates were Gram-negative, rod-shaped with flagella and could not grow in sulphide or in thiosulphate media, they were identified as members of the purple non-sulphur family *Rhodospirillaceae* (Cohen-Bazire *et al.* 1957; Staley *et al.* 1989). Non-sulphur bacteria are facultative anaerobes, heterotrophs and able to oxidize organic compounds photosynthetically or in the dark aerobically (Devlin & Barker 1971).

All isolates gave similar absorption spectra at 375, 590, 805 and 863 nm and these spectra were identical with those of bacteriochlorophyll *a* (Cohen-Bazire *et al.* 1957; Drews 1981; Pellerin & Gest 1983). The isolates hydrolysed gelatin, the unique property of photosynthetic bacteria in the genus *Rhodocyclus* (Buchanan *et al.* 1974; Watanabe *et al.* 1981; Imhoff *et al.* 1984; Staley *et al.* 1989).

Studies on the requirement of vitamins revealed that all four strains needed thiamine and biotin for growth, but not *p*-aminobenzoic acid or nicotinic acid. Slight growth was observed in the medium without vitamin B_{12}. *Rhodocyclus gelatinosus* requires thiamine and biotin for growth (Watanabe *et al.* 1981).

The nutrient requirements of the four isolates are presented in Table 1. All strains grew well in media with malate, pyruvate, acetate, propionate, glutamate, fructose or glucose, grew only slightly with sorbitol, tartrate, glycerol, or citrate and failed to grow on lactate, arginine, benzoic acid or thiosulphate. These results confirm that all four strains belong to *Rhodocyclus gelatinosus*.

Table 1. Carbon source requirement of four strains of photosynthetic bacteria isolated from seafood-processing wastewaters.

Carbon source	Growth*
Acetate	+ + +
Propionate	+ + +
Malate	+ + + +
Glutamate	+ + +
Sorbitol	+
Glycerol	+
Glucose	+ +
Fructose	+ +
Pyruvate	+ + + +
Citrate	+
Tartrate	+
Mannitol	+

* Cultures reached an absorbance value at 680 nm of 0.2 after incubation for 24 (+ + + +), 48 (+ + +), 72 (+ +) or 96 (+) h.

The effects of culture conditions on growth and pigment synthesis are shown in Table 2. In general, cultivation under aerobic-light or -dark conditions gave higher specific growth rates (0.04 to 0.06 h^{-1}), maximal biomass (1.7 to 2.3 g/l), cell yield (0.10 to 0.21 g cells/g COD), and protein content (36 to 48%) than when the strains were under anaerobic-light conditions (0.02 to 0.03 h^{-1}; 1.4 to 1.5 g/l; 0.13 to 0.18 g cells/g COD; 32 to 43%). No growth was observed when the strains were grown anaerobically in the dark. The higher biomass under aerobic conditions (both in the light and the dark) is due to the fact that under such conditions the photosynthetic bacteria use the Krebs cycle for the complete oxidation of the substrate.

Table 2. Performance of four isolated strains of *Rhodocyclus gelatinosus*, T6, R4, R5 and R7, cultivated in synthetic medium (G5), initial pH 7.0, after 35 h incubation under different conditions.

Parameter*	Aerobic-dark	Aerobic-light	Anaerobic-light
$\mu_{max}(h^{-1})$	0.04 to 0.05	0.05 to 0.06	0.02 to 0.03
X_{max} (g cell/l)	2.11 to 2.32	1.70 to 1.98	1.41 to 1.53
$Y_{x/s}$(g cell/g COD)	0.17 to 0.21	0.10 to 0.13	0.13 to 0.18
Protein (%)	40.0 to 48.3	36.1 to 45.7	32.7 to 42.8
Carotenoid (mg/g dry wt)	1.5 to 1.6	1.7 to 2.1	11.1 to 12.6
Bacteriochlorophyll (mg/g dry wt)	11.7 to 12.8	13.6 to 15.8	102.1 to 108.1
Final pH	8.50 to 8.53	8.40 to 8.52	7.42 to 7.68

μ_{max}—Specific growth rate; X_{max}—maximum dry cell wt; $Y_{x/s}$—cell yield.

Cell pigments were highest under anaerobic-light conditions, with carotenoid and bacteriochlorophyll contents of 11.8 to 12.6 and 102 to 108 mg/g dry cell wt compared with 1.5 to 2.1 and 11.7 to 15.9 mg/g dry cell wt obtained, respectively, under aerobic conditions. Under anaerobic-light conditions, the photosynthetic bacteria produce large amounts of pigments to store light energy for photosynthesis (Shipman *et al.* 1977). The culture broths were red in anaerobically-grown cultures and yellow in aerobically-grown cultures. The red and yellow carotenoids of *Rhodobacter sphaeroides* P47 growing under static-light conditions were identified as spheroidenone and spheroidene, respectively (Noparatnaraporn & Nagai 1986). The lower pigment concentrations observed under aerobic conditions was due to the effect of oxygen on the synthesis of carotenoid and bacteriochlorophyll; oxygen affects and inhibits the pigment synthesis and acts as bleaching agent (Cohen-Bazire *et al.* 1957). The yellow carotenoids of mutant strains of *Rhodopseudomonas spheroides* strain 24.1 were reported to be neurosporene and its dihydroxy derivative; an observed change in cell colour was due to the change in the ratio of the two carotenoid pigments (Cohen-Bazire *et al.* 1957).

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