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# Product Development and Derivatives Exploration by using Photosynthetic Bacteria

Yi-Fang Hung and Jinn-Tsyy Lai

***Abstract*—**Lycopene, which can be extracted from plants and is very popular for fruit intake, is restricted for healthy food development due to its high price. On the other hand, it will get great safety concerns, especially in the food or cosmetic application, if the raw material of lycopene is produced by chemical synthesis. In this project, we provide a key technology to bridge the limitation as mentioned above. Based on the abundant bioresources of BCRC (Bioresource Collection and Research Center, Taiwan), a promising lycopene output will be anticipated by the introduction of fermentation technology along with industry-related core energy. Our results showed that addition of tween 80(0.2%) and span 20 produced higher amount of lycopene. And piperidine, when was added at 48hr to the cultivation medium, could promote lycopene excretion effectively also.

***Keywords*—** photosynthetic bacteria, lycopene, tween80, Piperidine

## I. Introduction

PHOTOSYNTHETIC bacteria (PSB) are widely applied in aqui-culture, feed industry and wastewater treatment. Most of the researches on photosynthetic bacteria are focused on the improvement of hydrogen production [1] and the photo-synthesis mechanism, function of the pigments (e.g. carotenoids , lycopene ) in the metabolism pathway[2][3][4]. However, the accumulation of lycopene in *Rhodobacter sphaeroides* and *Rhodopseudomonas* sp. have a little information. There have been several reports on the bio-synthesis of carotenoids by photosynthetic bacteria like *R. sphaeroides* [5] and *Rhodospirillum sphaeroides* [6]Lycopene, $C_{40}H_{56}$, having a molecular weight of 536.9, is an important antioxidant among diverse carotenoids, and is a red-coloured intermediate within the β-carotene biosynthetic pathway [7]. It exhibits the function of clearing the free radicals, inducing cell junction communi- cation, regulation of tumor cells and other biological performances [8]-[10]. And lycopene also linked with reduced risk of prostate cancer [11]. Based on these advantages, carotenoids are promoted globally for exploring value added products to improve the good health of mankind. A recent report predicted ‘‘the world carotenoid market is expected to reach Euro 0.77 billionby 2010 as consumers continue to look for natural ingredients” [12]. As previous reports, several chemical agents will result in carotenogenesis in a number of microbial systems, especially hindrance of enzyme activity. These compounds, which include terpenes, ionones, amines, alkaloids, have been investigated systematically[13]-[16]. In this work, we attempt to enhance the production of lycopene using various surfactants and various lycopene cyclase inhibitors were also evaluated for their ability to accumulate lycopene.

Y.F. Hung, the Associate Research Scientist, Bioresource Collection and Research Center , Food Industry Research and Development Institute, Hsinchu, Taiwan. (phone: 886-3-5223191-518; fax:886-3-5224171; e-mail: hyf10@firdi.org.tw)
J.T. Lai, the Senior Research Scientist, Bioresource Collection and Research Center , Food Industry Research and Development Institute, Hsinchu, Taiwan. (Email: jtl@firdi.org.tw)

## II. Methods and Materials

*Medium sources*

D-glucose, fructose, sucrose, lactose, magnesium sulphate, sodium malate, sodium pyruvate, sodium fumarate, were purchased from Merck Ltd. Imidazole, nicotinic acid, creatinine and piperidine were purchased from Unionward corp. Sorbitan monolaurate LR (span 20), polyoxyethylene sorbitan mono laurate (tween 20), polyoxyethylene sorbitan monooleate (tween 80), were purchased from Merck Ltd. Standard lycopene powder 95% was procured from Merck Ltd.

*Microorganisms and culture conditions*

The *Rhodobacter sphaeroides* and *Rhodopseudomonas* sp. selected from BCRC , Hsinchu, Taiwan. Initial cultures were maintained on NS agar plate at 25 ± 2 ℃ for 5 days. After that , the plates were kept at 4 ℃, and thereafter sub-cultured were implemented every 20 days. NS medium for purple non-sulfur bacteria , contains yeast extract 0.05g, sodium malate 0.5 g, $NH_4Cl$ 0.1 g, $K_2HPO_4$ 1.0 g, NaCl 0.5g, Mineral salts solution ($FeSO_4 \cdot 7H_2O$ 0.01g, $CaCl_2$ 0.02g, $MnCl_2 \cdot 4H_2O$ 0.002g, $MgSO_4 \cdot 7H_2O$ 0.2g, $NaMoO_4 \cdot 2H_2O$ 0.001g ) on the base of 1L solution. Initial pH of the growth medium was maintained at 7.0-7.2. The culture was grown at a temperature of 30°C, and at light intensity of 2000- 3000 lux in anaerobic environment.

*Analysis of cell growth*

The growth of PSB was correlated with the increment of optical density at 660 nm. Along with monitoring the increaseabsorbance of cell broth, moreoverall experiments were carried out three tests. A standard curve of PSB cell concentration was determined between optical density and dry cell weight. And it was concluded an unit absorbance increaseis equivalent to 0.66 g dry cell weight per liter culture under experimental conditions.

*Effect of surface-active agents and carbon source on lycopene production*

A series of flasks with 50 ml of NS medium were supplemented with 2% surfactants, such as span 20, span 80, tween 20, tween 40, or tween 80 as well, after 48 hr incubation.. Another series of flasks containing 50 ml of NS medium were

added with 1% glucose, fructose, sucrose, and lactose, respectively, to examine the influence of lycopene production.

*Effect of lycopene cyclase inhibitors on the cell mass and lycopene production*

Influence of chemical inhibitors, such as imidazole, nicotinic acid, creatinine and piperidine, was explored under 100 , 500 , and 1000 ppm, severally. Both biomass growth and lycopene production were investigated and a siutable time will be probed for the addition of inhibitor with respect to lycopene production.

*Sample preparation and lycopene determination*

Sample (1 g) was extracted by using co-solvent (hexane: acetone: 1:4) containing 0.05% (w/v) butylated hydroxytoluene (BHT). The mixture was then agitated at 200 rpm in an orbital shaker for 30 min under room temperature. Then, the extract was filtered through a Whatman paper no. 4, and the residue was re-extracted twice, where the solvent volume was equal to the extractive medium. Moreover, the filtrate was added 10 ml of deionized water, and the mixture was shaken at 200 rpm for 5 min. The sample solution was finally conditioned for 5 min in a separating funnel for phase separation. The hexane layer was evaporated using a rotary evaporator and one milliliter of tetra- hydrogenfuran (THF) was used to dissolve the extract.Afterwards, the resulting solution was filtered through a 0.22 μm filter before injecting into the HPLC system. For HPLC assay, an analytical scale polymeric C30 (150 *4.6 mm, 3 μm) column (YMC Europe, Germany) was introduced. Whilethe mobile phase (0.8mL/minute) consisted of methanol (solvent A) and methyl tert-butyl ether (solvent B). Subsequently, the following gradient procedures were used: 1) Initial conditions 90% solvent A and 10% solvent B, 2) a 5-minute linear gradient to 75% solvent B, 3) a 20-minute linear gradient to 100% solvent B. The column temperature was kept at 40 ± 1°C, and 20μL injection volume was given for each trial.The flow rate of the mobile solution was 0.8 ml/min and the detective wavelength was set at 470 nm. Quantitative analysis was performed by plotting standard graph of powder lycopene 95% in the range 25–700 μg/ml.

*Evaluation of cell biomass*

The cell residue obtained after solvent extraction of pigments was filtered through Whatman no.1 filter paper under vacuum, along with distilled water washing thoroughly (twice), and dried at 55□ overnight.

## III. Results and Discussion

*Lycopene extraction*

Among various kinds of extraction solvent, the highest extraction efficiency of lycopene from cell pellets was obtained with a mixture of Hexane / Acetone (1:4 v/v) at 50℃ with nearly 100% efficency(TABLE I).

*Effect of surfactants on lycopene production*

The effect of surface-active agents on lycopene production of *Rhodopseudomonas* sp has not yet been fully investigated. And we evaluated the effect of different surface-active agents, such as span 20, span 80, tween 20, tween 40, and tween 80, respectively, at 0.2 % during the fermentation of *Rhodopseudomonas* sp. to enhance the excretion of lycopene. The result showed that tween 80 displayed the most promisingone of all the surface-active agents. It increased the yield of lycopene from 202 μg/L to 1180μg /L. And span20 also can enhance lycopene production from 202 μg/L to 1143μg /L. Other surface-active agents did not increase the yields significantly. (TABLE II)

TABLE I
Extraction efficiency of lycopene from wet cell pellets by different solvent or mixture at 50 ℃ for 30 min

| Solvent or mixture (v/v) | Efficiency [a,b] (%) |
|---|---|
| Methanol | 5 |
| Ethanol | 6 |
| Acetone | 32 |
| THF | 35 |
| Ethyl acetate | 26 |
| Hexane | 44 |
| Isopropanol | 23 |
| Hexane:Acetone (1:1) | 28 |
| Hexane:Acetone (2:1) | 28 |
| Hexane:Acetone (4:1) | 9 |
| Hexane:Acetone (1:2) | 60 |
| Hexane:Acetone (1:4) | 100 |

a.Values show extraction efficacy obtained after heating.
b.Data are expressed as mean of three replicates.

*Effect of carbon source on lycopene production*

Figure 1 showed the effect of carbon source on lycopene production of *Rhodopseudomonas* sp. has different presence. While evaluating the effect of different carbon source such as sucrose, glucose, fructose, and lactose,. 1 % addition of those substrates on the production of lycopene by *Rhodopseudomonas* sp. was evaluated. The results showed that sucrose was found to be the most effective compound of the trial carbon source. It increased the yield of lycopene from 202 μg/L to 929μg /L.

TABLE II
Effect of surface-active agents on lycopene production of *Rhodopseudomonas sp*

| Surface-active agents (0.2%) | Lycopene[a] (μg/L) |
|---|---|
| Span 20 | 1143 |
| Span 80 | 591 |
| Tween 20 | 475 |
| Tween 40 | 666 |
| Tween 80 | 1180 |
| Control | 202 |

a. Data are expressed as mean of three replicates.

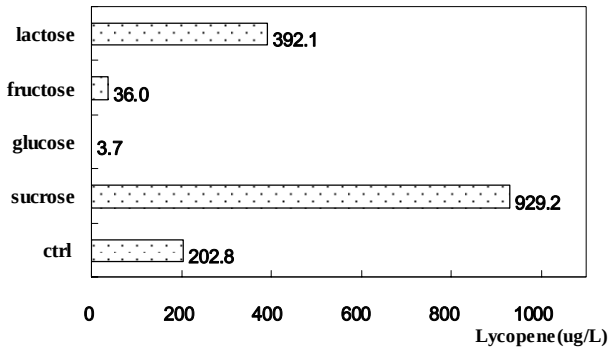


Fig.1.Effect of carbon source on lycopene production of *Rhodopseudomonas* s

Effect of lycopene cyclase inhibitors on biomass production and lycopene productionTo enhance the lycopene output using inhibitors of lycopene cyclase, the cultures which were treated with different inhibitors concentrations (100, 500 and 1000 ppm) were shown in Figure 2. These compounds enhanced lycopene formation by inhibiting the enzymes responsible for lycopene cyclization [17]. Piperidine at 1000 ppm added to the cultivation medium after 48 h culture of *Rhodopseudomonas* sp enhanced lycopene from 202 μg/L to 951μg /L. Low inhibitor concentration (100 ppm) did not significant inhibit the carotenoid biosynthetic pathway.

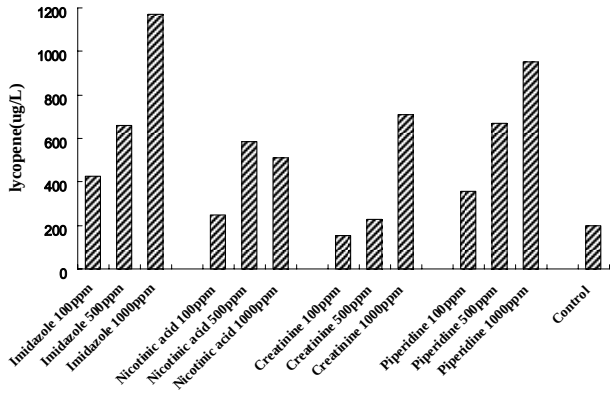


Fig.2.Effect of lycopene cyclase inhibitors on lycopene production of *Rhodopseudomonas* sp

## IV. Conclusion

The results showed some important aspects of the effect of stimulators on lycopene production. Both 0.2% of Tween 80 and Span 20 could obtain a higher lycopene production. And piperidine, added at 48hr to the cultivation medium also promoted lycopene excretion. In this study, hence, we could offer promising the processes of screening and mutation of photosynthetic bacteria, and a fermentation strategy for mass production as well.

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