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The Carotenoids Production from *Rhodopseudomonas* sp. OS33-UV13-5 Cultivated in Chitin Medium

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Abstract

In this work, the carotenoids (CD) production of *Rhodopseudomonas* sp. OS33-UV13-5 in the presence of different chitin forms such as chitin powder, chitin flake, squid pen and squid pen powder was studied for carbon source utilization, respectively. *Rhodopseudomonas* sp. OS33-UV13-5 showed the ability to degrade chitin. When *Rhodopseudomonas* sp. OS33-UV13-5 was cultivated in chitin medium containing 10 g/L of squid pen powder as a carbon source, the growth (bacteriochlorophylls content, Bchl) and CD were found at 6.32±0.75 Bchl U_{770nm} and 23.14±1.86 mg/L, respectively. Then, the optimized culture conditions were examined with various squid pen powder concentrations and light intensities. The results showed that maximum cell growth and CD production were 14.64±1.91 Bchl U_{770nm} and 45.87±3.85 mg/L, respectively, when cultivated in 30 g/L of squid pen powder under 1500 lux of light intensity at 40°C for 15 days. The CD production increased by about 2.02-fold. The *Rhodopseudomonas* sp. OS33-UV13-5 chitinase production displayed a maximum at 9.94±1.08 U/mL on day 9 of cultivation. The accumulation of N-acetyl glucosamine (NAG) was about 1.98±0.32 to 3.38±0.81 mg/L. Future research should emphasize on the fed-batch cultivation to enhance the CD production by maximizing chitin assimilation.

Keywords: Chitin, Carotenoids, *Rhodopseudomonas* sp., N-acetyl glucosamine

1. Introduction

The purple non-sulfur photosynthetic bacteria (PNSB) constitute a group of versatile organisms in which most exhibit four modes of metabolism: photoautotrophic, photoheterotrophic, chemoheterotrophic and chemoautotrophic, switching from one mode to another depending on the available conditions (Larimer et al., 2003). This metabolic versatility allows *Rhodopseudomonas palustris* to use light, inorganic, and organic compounds as its carbon and energy sources under anaerobic or aerobic conditions. *R. palustris*, which belongs to PNSB is widely distributed in nature, mainly in anaerobic water environments with sufficient light, such as lakes, soils, swamps, and the sea (Guan et al., 2017). Therefore, *R. palustris* contains photosynthetic pigments, Bchl, and CD to convert light energy into chemical energy by the process of anoxygenic photosynthesis (Pfennig, 1969). *R. palustris* is rich in various metabolites, contributing to its application in agriculture, aquaculture and livestock breeding as additives. Supplementation of *R. palustris* to aquaculture water can improve the immunity of aquatic organisms and prevent diseases (Zhou et al., 2010).

CD has been an important dietary supplement in the aquaculture industry for a long time because commercial aquatic animals cannot synthesize them by themselves. In aquaculture, the outstanding types of CD that have been the focus are β-carotene, zeaxanthin, canthaxanthin and astaxanthin because they enhance meat colors and help boost the immune system (Niu et al., 2014). CD are commonly included in the diets of salmonids, crustaceans, and other farmed fish primarily as pigments to enhance the desirable coloration of these cultured organisms

(García-Chavarría & Lara-Flores, 2013). Moreover, is considered a semi-essential nutrient that supports optimal survival and growth, even at relatively low dietary inclusion levels. Several studies have shown that fish fed diets containing CD exhibit significantly better growth and survival rates compared to those fed CD-free diets (Lim et al., 2018; Nakano, 2003). In fish, CD act as vitamin A precursors (Schiedt et al., 1985); significantly influences reproductive performance (Vassallo-Agius et al., 2001); function as potent antioxidants (Bell et al., 2000); enhance the immune system (Amar et al., 2003); and affect liver structure (Page et al., 2005). *R. palustris* normally accumulates CD series such as lycopene, rhodopin, rhodovibrin and spirilloxanthin (Mehrabi et al., 2001). Some researchers have shown that *R. palustris* can synthesize CD by using various carbon sources such as malate, succinate, fumarate, acetate, ethanol, lactate, propionate (Truper & Pfennig, 1981), raw starch (Krairak, 2023) as well as different light sources and intensities (Kuo et al., 2012). Krairak (2023) found that the PNSB mutant *Rhodopseudomonas* sp. OS33-UV13-5 was capable of growing in medium containing shrimp chitin as a carbon source. Marđetko et al. (2025) studied the cultivation of PNSB, *Rhodovulum adriaticum* DSM 2781, in chitin medium for Bchl-a production.

In previous research, CD production by *Rhodopseudomonas* sp. OS33-UV13-5 was studied using a raw starch medium (Krairak, 2023). However, *Rhodopseudomonas* sp. OS33-UV13-5 has chitin-digesting capability. Therefore, this study emphasizes the cultivation of *Rhodopseudomonas* sp. OS33-UV13-5 in chitin medium composed of chitin powder, chitin flake, squid pen and squid pen powder as carbon sources, respectively. The CD concentration was optimized for the maximum production under varying chitin concentrations and light intensities.

2. Materials and Methods

2.1 Chemical reagents and microorganism cultivation

The analytical-grade reagents used in this study were obtained from Himedia (India). Chitin powder and chitin flake were purchased from Himedia. Fresh squid (*Loligo* sp.) pen was purchased from Ang-Sira fishing market, Chonburi, Thailand. Prior to utilization, the squid pen was cleaned with distilled water to get rid of adherent proteins and soluble organic matter. Then it was allowed to dry at room temperature. The dried squid pen was cut into 1.0 cm x 1.0 cm pieces for using as squid pen carbon source. In the case of using squid pen powder as a carbon source, the dried squid pen was crushed into powder by a Waring blender 7010S (Waring Laboratory, USA) with a 250 µm screen sieve (Gilson, USA). The *Rhodopseudomonas* sp. OS33-UV13-5 (Krairak, 2023) used in this study was stored at 4°C as a stock culture. The chitin medium was modified from a starch medium (Krairak, 2023) by replacing starch with the following carbon sources: NAG and chitin, respectively. The inoculum was prepared by transferring 1 loopful of *Rhodopseudomonas* sp. OS33-UV13-5 into a chitin medium containing 1.0% NAG as the carbon source. The pre-culture was incubated at 40°C under 1000 lux of light intensity (incandescent lamp, 60W-Phillips®) for 7 days.

2.2 Types of chitin for *Rhodopseudomonas* sp. OS33-UV13-5 cultivation

The cell growth and CD production of *Rhodopseudomonas* sp. OS33-UV13-5 were examined by using carbon sources such as NAG, chitin powder, chitin flake, squid pen and squid pen powder, respectively. The 10 g/L of each carbon source was introduced into the chitin medium. 200 mL of chitin medium were placed in a 250-mL Duran bottle and sterilized at 121°C for 15 min. An inoculum of 1.0% was transferred into the chitin medium and incubated at 40°C under 1000 lux of light intensity for 15 days. Triplicate samples were examined to analyze Bchl content, NAG and CD concentrations.

2.3 Optimal light intensity for *Rhodopseudomonas* sp. OS33-UV13-5 cultivation

Effects of light intensity on cell growth and CD production from *Rhodopseudomonas* sp. OS33-UV13-5 were tested by using 30 g/L of squid pen powder and incubated at light intensities of 1000, 1500, and 2000 lux. The cultivation was carried out at 40°C for 15 days. Triplicate samples were examined to analyze Bchl content, NAG and CD concentrations.

2.4 The chitinase production

Chitinase production of *Rhodopseudomonas* sp. OS33-UV13-5 was studied in a chitin medium containing 30 g/L of squid pen powder. An inoculum of 1.0% was transferred to 200 mL of chitin medium and then incubated at 40°C under 1500 lux of light intensity for 15 days. Triplicate samples were examined to analyze Bchl content, NAG and chitinase activity.

2.5 Bchl and CD analysis

The cell concentration of *Rhodopseudomonas* sp. OS33-UV13-5 was examined by measuring Bchl content. The extracted Bchl from *Rhodopseudomonas* sp. OS33-UV13-5 was analyzed by the method of Cohen-Bazire et al. (1957). The cell pellet, which was retrieved by centrifugation at 10,000 *g* for 15 min, was used to extract by mixing with a solvent mixture of acetone and methanol (7:2, v/v) for 2 hours. Then, the extracted mixture was centrifuged at 10,000 *g* for 15 min. The supernatant was collected and measured at 770 nm (OD770) for Bchl content. For CD analysis, the absorbance at 480 nm (OD_{480}) was measured, and then the concentration of CD was determined using the Beer-Lambert law. The extinction coefficient value used for CD was was 25 $m^3.mol^{-1}.cm^{-1}$ (Lopez-Romero et al, 2020).

2.6 NAG measurement and chitinase assay

The activity of chitinase was measured colorimetrically by detecting the amount of NAG liberated from the degradation of the substrate, colloidal chitin. The reaction mixture containing 1.0 mL of 1.0 % (w/v) colloidal chitin (in 0.02 M phosphate buffer, pH 7.0) and 1.0 mL of diluted enzyme solution was incubated for 60 min at 40°C, followed by the addition of 3,5-dinitrosalicylic acid (DNS) reagent and boiled for 10 min. After cooling, the developed color, indicating the amount of released NAG, was measured at 530 nm (Wang et al., 2009). The amount of NAG was calculated from the standard curve of NAG. One unit of chitinase activity was defined as the amount of enzyme that liberated 1 µmol of NAG per minute.

2.7 Statistical analysis

All experiments were carried out in triplicate and mean ± SD values are reported. Data were analyzed using ANOVA, followed by Duncan's multiple range test, using the SPSS (Statistical Package for Social Sciences, SPSS Inc., USA) software package.

3. Results and Discussion

3.1 Effects of the carbon source on cell growth and CD production

The growth of *Rhodopseudomonas* sp. OS33-UV13-5, in terms of Bchl content, in a medium containing NAG, chitin powder, chitin flake, squid pen and squid pen powder, respectively, is shown in Figure 1.

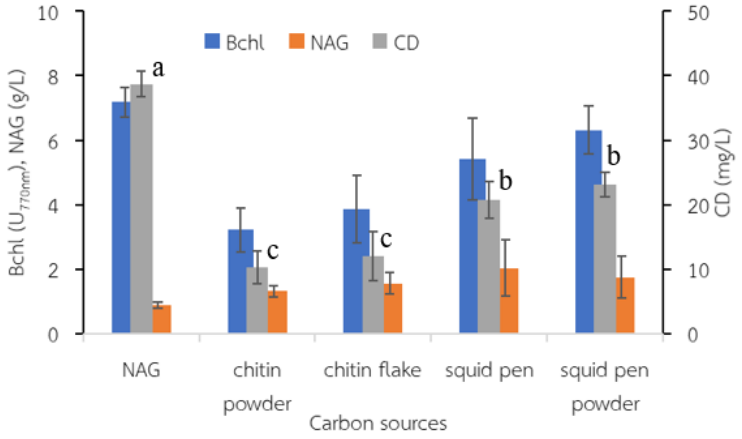

Figure 1. The growth (Bchl content) and CD production of *Rhodopseudomonas* sp. OS33-UV13-5 cultivated in NAG, chitin powder, chitin flake, squid pen and, and squid pen powder as carbon sources. The incubation was performed at 40°C under 1000 lux of light intensity for 15 days. The same letters indicate no significant difference (p<0.05).

The maximum growth of *Rhodopseudomonas* sp. OS33-UV13-5 was found at 7.18±0.45 Bchl U_{770nm} when NAG was used as the carbon source. Among the tested carbon sources, only NAG was a water-soluble compound that could be easily assimilated by *Rhodopseudomonas* sp. OS33-UV13-5. This result indicated that 10 g/L of NAG not only did not inhibit growth but also promoted the CD production. When squid pen powder and squid pen were used as carbon sources, *Rhodopseudomonas* sp. OS33-UV13-5 exhibited cell growth levels of 5.41±1.27 and 6.32±0.75 Bchl U_{770nm} and CD production of 20.78±2.79 mg/L and 23.14±1.86 mg/L, respectively. However, *Rhodopseudomonas* sp. OS33-UV13-5 presented the lowest growth and CD production in the medium containing chitin powder and chitin flake as carbon sources. The results indicated that *Rhodopseudomonas* sp. OS33-UV13-5 could more easily digest squid pen and squid pen powder than chitin powder and chitin flake. This is likely due to the different chitin polymorphs present: squid pen and squid pen powder contain β-chitin, which is composed of parallel chains of heteropolymers with weak intra-sheet hydrogen bonds. In contrast, chitin powder and chitin flake are typically prepared from shrimp shells through deproteinization and demineralization, resulting in α-chitin, which is composed of tightly compacted parallel and antiparallel chains of heteropolymer with strong intra-sheet hydrogen bonds (Minke & Blackwell, 1978). Therefore, squid pen and squid pen powder were more susceptible to enzymatic digestion by *Rhodopseudomonas* sp. OS33-UV13-5. NAG was released via enzymatic hydrolysis from these four carbon sources and was finally assimilated for cell growth and CD formation (Lavall et al., 2007; Minke & Blackwell, 1978). From the results of this experiment, squid pen powder was the most suitable carbon source for growth and CD production by *Rhodopseudomonas* sp. OS33-UV13-5.

3.2 Effects of the squid pen powder concentration on growth and CD production

The chitin medium using squid pen powder as a carbon source was prepared at concentrations of 10, 20, 30, 40 and 50 g/L, respectively. An inoculum of 1.0% was transferred to 200 mL of chitin medium. The cultivation was carried out at 40°C under 1000 lux of light intensity for 15 days, results are shown on Figure 2.

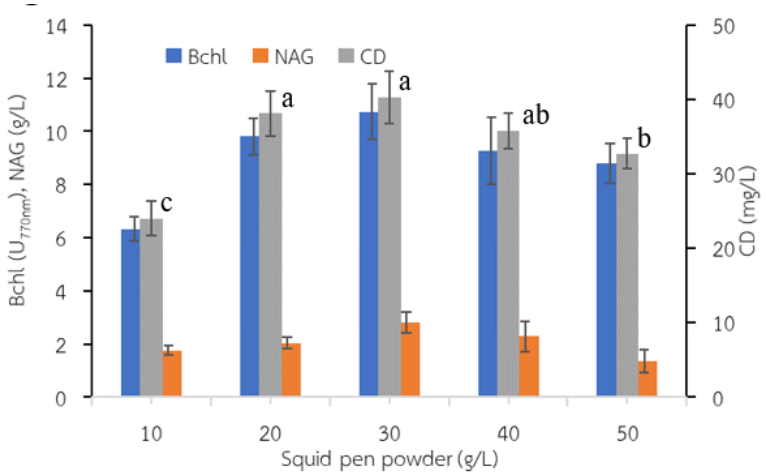

Figure 2. The growth (Bchl content) and CD production of *Rhodopseudomonas* sp. OS33-UV13-5 cultivated in chitin medium with varying concentrations of squid pen powder. The incubation was performed at 40°C under 1000 lux of light intensity for 15 days. The same letters indicate no significant difference (p<0.05).

The results showed that cultivation in chitin medium supplemented with 30 g/L of squid pen powder as the carbon source resulted in the maximum growth and CD production at 10.74±1.05 Bchl U_{770nm} and 40.27±3.75 mg/L, respectively. Additionally, the NAG concentration was found to be 2.81 g/L. However, at squid pen powder concentrations higher than 40 g/L, a reduction in both cell growth and CD production was observed. Although the CD production by *Rhodopseudomonas* sp. OS33-UV13-5 cultivated in squid pen powder medium at concentrations of 20 g/L and 30 g/L was statistically insignificant, the cell concentration was highest in the 30 g/L medium. This suggests that resource limitations affected both cell growth and CD production. These limitations may include insufficient light intensity and limited nutrient availability. In terms of light, shading effects likely occurred at high cell densities, reducing the effective light reaching the cells. Regarding nutrients, Liu & Wu

(2007) suggested that a high C/N ratio promotes CD production. Therefore, the 30 g/L squid pen powder medium might offer strong potential to maximize CD production efficiency. Investigating the optimal light intensity could further enhance CD production under these conditions.

3.3 Effects of the light intensity on growth and carotenoid production

A squid pen powder medium at 30 g/L was studied for cell growth and CD production by *Rhodopseudomonas* sp. OS33-UV13-5. An inoculum of 1.0% was transferred to 200 mL of chitin medium. The cultivation was carried out at 40°C under light intensities of 1000, 1500, and 2000 lux for 15 days. The results are shown in Figure 3.

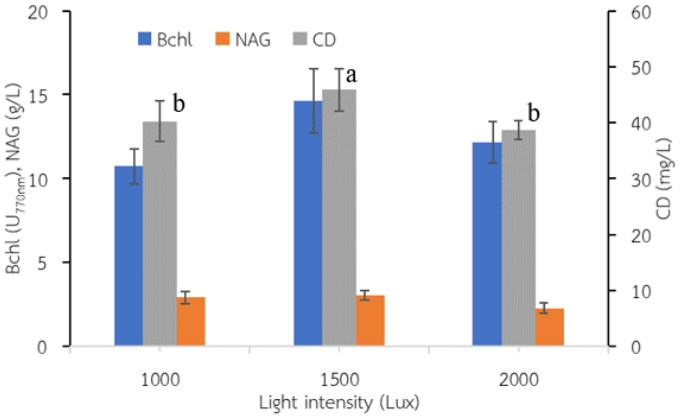

Figure 3. The growth (Bchl content) and CD production of *Rhodopseudomonas* sp. OS33-UV13-5 cultivated in a medium containing 30 g/L squid pen powder as the carbon source. The incubation was performed at 40°C under light intensities of 1000, 1500, and 2000 lux for 15 days. The same letters indicate no significant difference (p<0.05).

It was indicated that a light intensity of 1500 lux resulted in the maximum cell growth and CD production at 14.64±1.91 Bchl U_{770nm} and 45.87±3.85 mg/L, respectively. The CD production increased approximately 2.02-fold. A light intensity of 1500 lux provided the optimal conditions for photosynthesis, which is associated with the availability of cofactors involved in the mevalonate (MVA) pathway and the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway for carotenoid biosynthesis (Alper et al., 2005). This finding suggests that a light intensity of 1500 lux is favorable for the growth of *Rhodopseudomonas* sp. OS33-UV13-5 and CD production in a medium containing 30 g/L squid pen powder, likely because the cells harvested more energy at 1500 lux and received sufficient nutrients. Schagerl and Müller (2006) explained that CD production had two functions. it acts as a light-harvesting pigment when exposed to weak light and, on the other, it plays an important role in photoprotection when exposed to intense light. In contrast, at 2000 lux of light intensity, both cell growth and CD production were reduced. The excessive light intensity might lead to oxidative damage. To minimize the excitation of photosynthetic reaction centers, most photosynthetic organisms rapidly dissipate excess light energy as harmless heat (Li et al., 2000).

3.4 Chitinase production during *Rhodopseudomonas* sp. OS33-UV13-5 cultivation in squid pen powder medium

Rhodopseudomonas sp. OS33-UV13-5 was cultivated in a medium containing 30 g/L squid pen powder as the carbon source and incubated at 40°C under 1500 lux of light intensity for 15 days. The culture was examined for cell growth, chitinase production and NAG concentration. The results are shown in Figure 4.

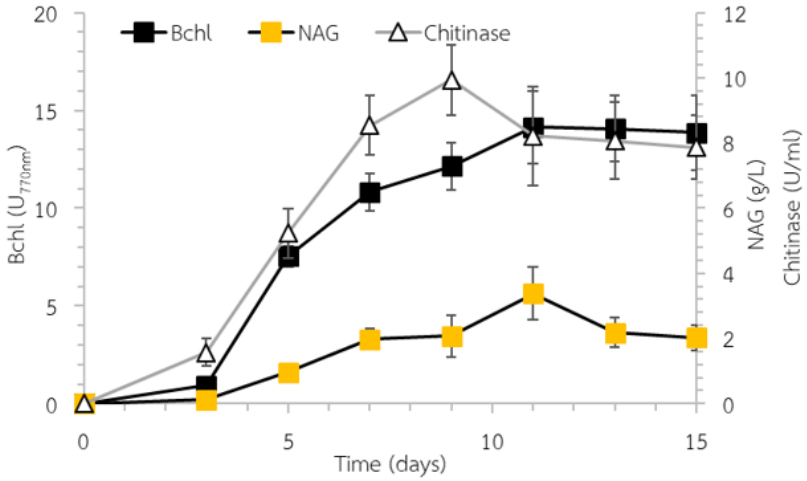

Figure 4. The growth (Bchl content) and chitinase production of *Rhodopseudomonas* sp. OS33-UV13-5 cultivated in a medium containing 30 g/L squid pen powder as the carbon source. The incubation was performed at 40°C under 1500 lux of light intensity for 15 days.

It was found that cell growth and chitinase production increased simultaneously. The maximum chitinase production (9.94±1.08 U/mL) was reached on day 9 of cultivation. After that, chitinase activity gradually decreased until day 15. The highest cell growth (14.14±1.84 Bchl U_{770nm}) was observed on day 11 and remained stable until the end of the cultivation period. In addition, the maximum NAG concentration was observed at 3.38±0.81 mg/L on day 11 of cultivation. Chitin was partially depolymerized into oligosaccharides or completely digested into its monomer, NAG, by chitinase (Ramírez et al., 2010). NAG was then assimilated into cellular metabolism. This process might be related to the accumulation of acetyl-CoA or pyruvic acid, which was ultimately converted into isopentenyl pyrophosphate (IPP) and dimethylallyl diphosphate (DMAPP) via the mevalonate (MVA) pathway or the methylerythritol 4-phosphate (MEP) pathway (Li et al., 2019). Both IPP and DMAPP play essential roles as intermediates in terpenoid (isoprenoid) biosynthesis, and their formation is considered the first step in the carotenogenesis pathway (Raposo et al., 2015). However, residual squid pen powder was still observed at the end of cultivation. This indicates that chitin utilization was incomplete. This might be due to limitations in certain substrate components, such as nitrogen sources and other essential nutrients. Future experiments should focus on improving chitin utilization by supplementing nitrogen sources in a fed-batch cultivation system.

4. Conclusions

Rhodopseudomonas sp. OS33-UV13-5 is a mutant obtained through UV mutagenesis of the wild-type *Rhodopseudomonas* sp. OS33.This mutant demonstrated the ability to grow in media containing chitin derived from shrimp shells and squid pens as carbon sources. Among these, squid pen chitin was suitable for cell growth (measured as Bchl content) and CD production. Squid pen contains β-chitin, which is more susceptible to enzymatic digestion by *Rhodopseudomonas* sp. OS33-UV13-5 chitinase than the α-chitin present in the shrimp shell. When cultivated in a chitin medium containing 30 g/L of squid pen powder as a carbon source and incubated under 1500 lux of light intensity, *Rhodopseudomonas* sp. OS33-UV13-5 produced a maximum CD concentration of 45.87±3.85 mg/L. Future research should focus on fed-batch cultivation to enhance CD production by maximizing chitin assimilation. Therefore, it is necessary to study and determine the optimal nutrient feeding strategy to increase both CD production and cell biomass. *Rhodopseudomonas* sp. OS33-UV13-5 has the potential to become a biosource of CD capable of meeting global demands across aquaculture, pharmaceutical, food, beverage, poultry, cosmetic, and other industries.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

References

Alper, H., Jin, Y.-S., Moxley, J. F., & Stephanopoulos, G. (2005). Identifying gene targets for the metabolic engineering of lycopene biosynthesis in *Escherichia coli*. *Metabolic Engineering*, *7*(3), 155–164. https://doi.org/10.1016/j.ymben.2004.12.003

Amar, E. C., Kiron, V., Satoh, S., & Watanabe, T. (2003). Enhancement of innate immunity in rainbow trout (*Oncorhynchus mykiss* Walbaum) associated with dietary intake of carotenoids from natural products. *Fish and Shellfish Immunology*, *16*, 527–537.

Bell, J. G., McEvoy, J., Tocher, D. R., & Sargent, J. R. (2000). Depletion of α-tocopherol and astaxanthin in Atlantic salmon (*Salmo salar*) affects autoxidative defense and fatty acid metabolism. *The Journal of Nutrition*, *130*, 1800–1808.

Cohen-Bazire, G., Sistrom, W. R., & Stanier, R. Y. (1957). Kinetic studies of pigment synthesis by non-sulfur purple bacteria. *Journal of Cellular Physiology*, *49*, 25–68.

García-Chavarría, M., & Lara-Flores, M. (2013). The use of carotenoid in aquaculture. *Research Journal of Fisheries and Hydrobiology*, *8*(2), 38–49.

Guan, C., Ji, Y., Hu, J., Hu, C., Yang, F., & Yang, G. (2017). Biotransformation of rutin using crude enzyme from *Rhodopseudomonas palustris*. *Current Microbiology*, *74*, 431–436. https://doi.org/10.1007/s00284-017-1204-3

Krairak, S. (2023). Production of coenzyme Q10 and carotenoid by cultivation of *Rhodopseudomonas* sp. OS33-UV13-5 using raw starch as carbon source. *Journal of Science Ladkrabang*, *32*(2), 136–151.

Kuo, F.-S., Chien, Y.-H., & Chen, C.-J. (2012). Effects of light sources on growth and carotenoid content of photosynthetic bacteria *Rhodopseudomonas palustris*. *Bioresource Technology*, *113*, 315-318. https://doi.org/10.1016/j.biortech.2012.01.087

Larimer, F. W., Chain, P., Hauser, L., Lamerdin, J., Malfatti, S., Do, L., Land, M. L., Pelletier, D. A., Beatty, J. T., Lang, A. S., Tabita, F. R., Gibson, J. L., Hanson, T. E., Bobst, C., Torres, J. L. T., Peres, C., Harrison, F. H., Gibson, J., & Harwood, C. S. (2003). Complete genome sequence of the metabolically versatile photosynthetic bacterium *Rhodopseudomonas palustris*. *Nature Biotechnology*, *22*, 55–61.

Lavall, R. L., Assis, O. B., & Campana-Filho, S. P. (2007). β-Chitin from the pens of *Loligo* sp.: Extraction and characterization. *Bioresource Technology*, *98*(13), 2465–2472.

Li, M., Hou, F., Wu, T., Jiang, X., Li, F., Liu, H., Xian, M., & Zhang, H. (2019). Recent advances of metabolic engineering strategies in natural isoprenoid production using cell factories. *Natural Product Reports*, *37*(1), 80–99.

Li, X. P., Bjorkman, O., Shih, C., Grossman, A. R., Rosenquist, M., Jansson, S., & Niyogi, K. K. (2000). A pigment-binding protein essential for regulation of photosynthetic light harvesting. *Nature*, *403*, 391–395.

Lim, K. C., Yusoff, F. M., Shariff, M., & Kamarudin, M. S. (2018). Astaxanthin as feed supplement in aquatic animals. *Reviews in Aquaculture*, *10*(3), 738–773.

Liu, Y. S., & Wu, J. Y. (2007). Optimization of cell growth and carotenoid production of *Xanthophyllomyces dendrorhous* through statistical experiment design. *Biochemical Engineering Journal*, *36*(2), 182–189. https://doi.org/10.1016/j.bej.2007.02.014

Lopez-Romero, J., Salgado-Manjarrez, E., Torres, L., & Garcia-Pena, E. I. (2020). Enhanced carotenoid production by *Rhodopseudomonas palustris* ATCC 17001 under low light conditions. *Journal of Biotechnology*, *323*, 159–165. https://doi.org/10.1016/j.jbiotec.2020.08.007

Marđetko, N., Kolakušić, A., Trontel, A., Novak, M., Pavlečić, M., Dobrinčić, A., Tominac, V. P., & Šantek, B. (2025). Usage of the fungus *Mucor indicus* and the bacterium *Rhodovulum adriaticum* in a biorefinery system for biochemical production on grass hydrolysates. *Polymers*, *17*(3), 369. https://doi.org/10.3390/polym17030369

Mehrabi, S., Ekanemesang, U. M., Aikhionbare, F. O., Kimbro, K. S., & Bender, J. (2001). Identification and characterization of *Rhodopseudomonas* spp., a purple, non-sulfur bacterium from microbial mats. *Biomolecular Engineering*, *18*, 49–56.

Minke, R., & Blackwell, J. (1978). The structure of α-chitin. *Journal of Molecular Biology*, *120*(2), 167–181.

Nakano, T. (2003). Microorganisms. In H. Nakagawa & M. Sato (Eds.), *Micronutrients and health of cultured fish* (pp. 95-106). Koseisha Koseikaku.

Niu, J., Wen, H., Li, C.-H., Liu, Y.-J., Tian, L.-X., Chen, X., Huang, Z., & Lin, H.-Z. (2014). Comparison effect of dietary astaxanthin and β-carotene in the presence and absence of cholesterol supplementation on growth performance, antioxidant capacity and gene expression of *Penaeus monodon* under normoxia and hypoxia condition. *Aquaculture*, *422-423*, 8–17. https://doi.org/10.1016/j.aquaculture.2013.11.013

Page, G. I., Russell, P. M., & Davies, S. J. (2005). Dietary carotenoid pigment supplementation influences hepatic lipid and mucopolysaccharide levels in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part B*, *142*, 398–402.

Pfennig, N. (1969). *Rhodopseudomonas acidophila*, sp. n., a new species of the budding purple nonsulfur bacteria. *Journal of Bacteriology*, *99*(2), 597–602.

Ramírez, M. Á., Rodriguez, A. T., Alfonso, L., & Peniche, C. (2010). Chitin and its derivatives as biopolymers with potential agricultural applications. *Biotecnología Aplicada*, *27*, 270–276.

Raposo, M. F. J., De Morais, A. M. M. B., & De Morais, R. M. S. C. (2015). Carotenoids from marine microalgae: A valuable natural source for the prevention of chronic diseases. *Marine Drugs*, *13*(8), 5128–5155. https://doi.org/10.3390/md13085128

Schagerl, M., & Müller, B. (2006). Acclimation of chlorophyll a and carotenoid levels to different irradiances in four freshwater cyanobacteria. *Journal of Plant Physiology*, *163*(7), 709–716. https://doi.org/10.1016/j.jplph.2005.09.015

Schiedt, K., Leuenberger, F. J., Vecchi, M., & Glinz, E. (1985). Absorption, retention and metabolic transformation of carotenoids in rainbow trout, salmon and chicken. *Pure and Applied Chemistry*, *57*(5), 685–692.

Truper, H. G., & Pfennig, N. (1981). Characterization and identification of the anoxygenic phototrophic bacteria. In M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, & H. G. Schlegel (Eds.), *The prokaryotes: A handbook on habitats, isolation, and identification of bacteria* (pp. 299-312). Springer-Verlag Berlin Heidelberg.

Vassallo-Agius, R., Imaizumi, H., Watanabe, T., Yamazaki, T., Satoh, S., & Kiron, V. (2001). The influences of astaxanthin supplemented dry pellets on spawning of striped jack. *Fisheries Sciences*, *67*, 260–270.

Wang, S.-L., Yang, C.-W., Liang, T.-W., Peng, J.-H., & Wang, C.-L. (2009). Degradation of chitin and production of bioactive materials by bioconversion of squid pens. *Carbohydrate Polymer*, *78*, 205–212. https://doi.org/10.1016/j.carbpol.2009.03.020

Zhou, X., Tian, Z., Wang, Y., & Li, W. (2010). Effect of treatment with probiotics as water additives on tilapia (*Oreochromis niloticus*) growth performance and immune response. *Fish Physiology and Biochemistry*, *36*(3), 501–509. https://doi.org/10.1007/s10695-009-9320-z