Vietnam Journal of Marine Science and Technology; Vol. 20, No. 4; 2020: 457–462
DOI: https://doi.org/10.15625/1859-3097/15788
http://www.vjs.ac.vn/index.php/jmst

Process of extraction and isolation of canthaxanthin from saline bacteria biomass *Paracoccus carotinifaciens* VTP20181 isolated in Vietnam

Dang Viet Anh[1], Tran Quoc Toan[1,*], Le Xuan Duy[1], Nguyen Manh Dat[2], Do Thi Thuy Le[2], Tran Thi Thuy Ha[3], Do Van Thinh[3], Le Van Khoi[3], Pham Quoc Long[1]

[1]*Institute of Natural Products Chemistry, VAST, Vietnam*
[2]*Food Industry Research Institute, Hanoi, Vietnam*
[3]*Research Institute for Aquaculture No. 1, Bac Ninh, Vietnam*
[*]E-mail: tranquoctoan2010@gmail.com

Received: 16 September 2020; Accepted: 30 November 2020

Abstract
Canthaxanthin is a natural carotenoid, which can be biosynthesized from several different bacterial strains and exhibits a wide range of biological benefits such as antioxidant, immune booster, and vascular stabilization. Canthaxanthin is widely used in the food and pharmaceutical industries as a natural colourant, and its production from natural sources has been the topic of growing interest. In this study, we established the process of extracting, enriching and isolating canthaxanthin from the post-fermentation biomass of the saline bacteria *Paracoccus carotinifaciens* VTP20181 isolated in Vietnam at laboratory scale. This process consists of 5 steps: Dry biomass generation, dry biomass extraction, removal of saturated fat, fast column chromatography and slow column chromatography, canthaxanthin purification. Canthaxanthin compound is obtained with 98% purity. Canthaxanthin extraction and purification efficiency of the whole process reached 55.5%.

Keywords: Canthaxanthin, *Paracoccus carotinifaciens* VTP20181, extraction process.

Citation: Dang Viet Anh, Tran Quoc Toan, Le Xuan Duy, Nguyen Manh Dat, Do Thi Thuy Le, Tran Thi Thuy Ha, Do Van Thinh, Le Van Khoi, Pham Quoc Long, 2020. Process of extraction and isolation of canthaxanthin from saline bacteria biomass *Paracoccus carotinifaciens* VTP20181 isolated in Vietnam. *Vietnam Journal of Marine Science and Technology*, *20*(4), 457–462.

INTRODUCTION

Special attention is being paid to natural pigments that replace beta-carotene derived synthetic pigments such as canthaxanthin, astaxanthin, lycopene, lutein with many biological activities such as antioxidant, colouring, and resistance enhancement both in research and commerce [1, 2]. Experimental studies and clinical evaluation of beta carotene and its derivatives such as canthaxanthin, astaxanthin, zeaxanthin, lutein have demonstrated the effects of this group on customer health. Carotenoids act as antioxidants, remove free radicals, strengthen cell walls as well as protect cells, thereby enhancing immunity, anti-tumour, support in treating cardiovascular disease and obesity [3–6]. Many surveys show that the most popular and most economically valuable fields of application for these pigments are food, pharmaceutical, cosmetic and livestock industries. The total value of carotene derived products worldwide in 2015 reached 1.21 billion USD, increasing gradually 3–5% each year. By 2025, the commercial value of these products is estimated at USD 2 billion [7].

Canthaxanthin (4,4'-diketo-β-carotene) is a carotenoid whose polyene backbone structure is more stable in the cell than active compounds with a single polyene backbone structure, thus providing a better antioxidant effect [8–10]. This compound is found in several plants, crayfish, salmon, birds, fungi, marine algae and bacteria. Canthaxanthin was discovered for the first time in an edible fungus called *Paracoccus carotinifaciens* [11, 12]. Canthaxanthin can be obtained from biosynthesis or chemical synthesis. In recent years, canthaxanthin isolated from microbiological sources has developed dramatically due to restrictions on the use of chemically synthesized carotenoids in the food and pharmaceutical industries. Compared with chemically synthesized canthaxanthin, microorganism-extracted canthaxanthin is of importance due to its safety and does not depend on factors such as geography, season.

The group of microorganisms that contain important canthaxanthin are bacteria (e.g: *Dietzia natronolimnaea* HS-1, *Micrococcus roseus* and *Rhodococcus maris*) and green microalgae (*Clorococcum* sp. MA-1, *Chlorella emersonii*, *Chlorellazofingiensis* and *Dictyococcus cinnabarinus*). In which, canthaxanthin synthesized from *D. natronolimnaea* HS-1 takes more than 90% of total obtained carotenoid [13]. According to Razavi et al., canthaxanthin is the dominant pigment in this bacterium. When batch cultured on fermentation equipment, canthaxanthin content reached 2.87 mg/l and when batch fermented with additional medium, canthaxanthin content increased to 13.17 mg/l [14]. Currently, in Vietnam, several strains capable of producing high content of canthaxanthin have also been isolated such as *Staphyloccocus* CNTP 4191, *Staphyloccocus* CNTP 4192, *Haloferax alexandrinus* NBRC 16590 and *Paracoccus carotinifaciens* VTP20181.

Based on the information above, the important role of the extraction and isolation of canthaxanthin from microorganisms and their biomass in the food and pharmaceutical industries is proven. In this study, we present the process of extraction and isolation of canthaxanthin at laboratory scale from biomass of bacteria *Paracoccus carotinifaciens* VTP20181 isolated in Vietnam.

TECHNOLOGICAL PROCESS OF EXTRACTING AND ISOLATING CANTHAXANTHIN FROM BIOMASS OF BACTERIA *Paracoccus carotinifaciens* VTP20181

Material

Biomass of the bacterium *Paracoccus carotinifaciens* VTP20181 obtained after fermentation was provided by the Food Industry Research Institute. Biomass was refrigerated (-40^{o}C) to prepare for extraction. The biomass solution is red brown, the moisture content is 80% and the canthaxanthin content is 0.3 mg/g of wet biomass.

Equipment, solvents and chemical reagents

High-pressure liquid chromatograph system, analysis column C18 (4.6 mm × 250 mm, particle size 5 µm), detector PDA or UV-Vis, wavelength 475 nm, pump LC-20AD, solvent channel B: methanol (0.1% acetic acid), solvent channel A: acetonnitrile.

Column chamber temperature 30^{o}C, flow rate 1 ml/min, injection volume 20 µl, column pressure 176 kgf/cm^{2}.

Rotary evaporator EYELA N-1200 A, magnetic stirrer, Hettich centrifuge, chromatographic column, vacuum pump, Buchner filter hopper, UP2000Ht probe ultrasound.

Solvents, chemical reagents: liquid nitrogen, argon gas, distilled water, acetone, n-hexane, methanol, acetonitril, glycerol monostearate (GMS), urea, 2 times distilled water.

Research diagram

The research process diagram is shown in figure 1 including 5 main steps: Dry biomass generation, total extract collection, fat removal using urea crystallization method, flash column chromatography to collect canthaxanthin-rich inoculants and column chromatography to obtain purified canthaxanthin.

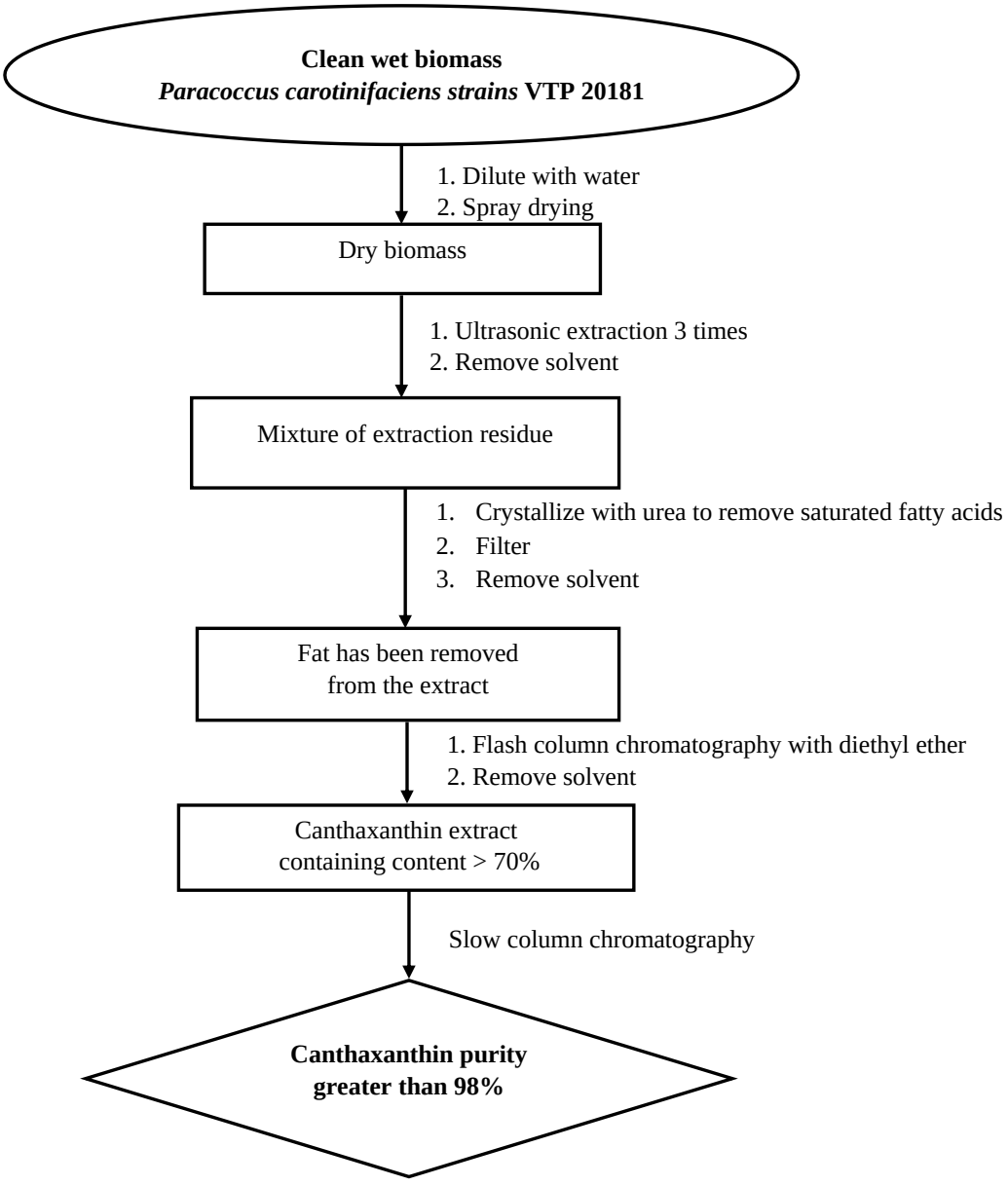

Figure 1. Diagram of the research process for extracting and isolating canthaxanthin

Experimental process

Dry biomass generation

About 5,000 g of wet biomass was dissolved with water at the ratio (1/2, w/w), 5% maltodextrin carrier was added to the wet biomass weight and stirred to create a homogeneous mixture. Spray drying was carried out to collect dry biomass. The technology parameters of the spray drying process are as follows: inlet and outlet hot air temperature is 1,650^{o}C/850^{o}C; The refuelling rate is 2.5 litres/hour, the rotation speed is 20,000 rpm. 1,250 g of dry biomass flowing powder was obtained with moisture 6%.

Extract of dry biomass

1,000 g of dry biomass was extracted using ultrasound with mixture solvent (ethanol 96 + 0.5% glycerol monostearate (GMS)) with solvent/material ratio (v/w) of 5/1. The extraction was repeated 3 times. Ultrasonic wattage is 120 W, ultrasonic time is 90 minutes/extraction, extraction temperature is 30^{o}C. The extracts were combined and purified using a Buchner funnel. Solvents were evaporated to obtain 45.5 g of total extract containing canthaxanthin.

Removal of saturated fat

40 g of total extract containing canthaxanthin and saturated fatty acids were crystallized with saturated urea in ethanol 96% according to the following technological parameters: the ratio of ethanol/total fatty acids 6/1 (v/w), urea/total fatty acids ratio 3.5/1 (w/w), crystallization temperature 0^{o}C, crystallization time 12 h. Filtration was carried out and crystallization was removed to obtain an aqueous solution. Solvent was evaporated to obtain 38.2 g of fat removed total extract.

Flash column chromatography

Flash column chromatography was performed using diethyl ether solvent. 35 g of total extract was diluted with ethanol and mixed with silica gel. The mixture was subjected to column chromatography, eluted with diethyl ether. The ratio of solvent/volume of silica gel was 3/1 (v/v). Solvents were evaporated to obtain 2.1 g of an extract rich in canthaxanthin.

Column chromatography, canthaxanthin purification

1.1 g of an extract was dissolved with analytical ethanol, then mixed with silica gel. The mixture was subjected to column chromatography, eluted with n-hexane/acetone (6/1 → 3/1, v/v) to give 6 fractions C1 → C6. The presence of canthaxanthin in fractions was checked using thin layer chromatography (TLC). The fractions containing pure canthaxanthin were collected, evaporated to obtain 345 mg of pure canthaxanthin. The purity was evaluated using high performance liquid chromatography (HPLC) and its physicochemical data was compared. Canthaxanthin extraction and purification efficiency of the whole process reached 55.5%. Canthaxanthin purity reached over 98%.

The physicochemical parameters of canthaxanthin

The physicochemical parameters of this compound were determined using different physical and chemical methods as follows:

ESI-MS mass spectrometry: m/z = 565 [M + H]$^{+}$;

Molecular formula: $C_{40}H_{52}O_2$;

State: red solid;

Melting point: 211–213^{o}C;

Maximum absorption at the wavelength λ_{max} 470 nm;

Solubility: insoluble in water, well soluble in acetone, chloroform.

Nuclear magnetic resonance spectrum was determined on a 500 MHz Bruker nuclear magnetic resonator at the Institute of Chemistry - Vietnam Academy of Science and Technology.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.86 (2H, m, H-2, 2'), 2.51 (2H, m, H-3, 3'), 6.25 (2H, m, H-7, 7'), 6.36 (2H, d, H-8, 8'), 6.27 (2H, m, H-10, 10'), 6.68 (2H, m, H-11, 11'), 6.40 (2H, d, H-12, 12'), 6.29 (2H, m, H-14, 14'), 6.65 (2H, m, H-15, 15'), 1.20 (6H, s, H-16, 16'), 1.20 (6H, s, H-17, 17'), 1.88 (6H, s, H-18, 18'), 2.00 (6H, s, H-19, 19'), 2.18 (6H, s, H-20, 20').

^{13}C NMR: δ 198.7 (C=O), 160.9 (C-6, 6'), 141.1 (C-8, 8'), 139.3 (C-12, 12'), 136.6 (C-13, 13'), 134.8 (C-9, 9'), 134.3 (C-10, 10'), 133.6 (C-14, 14'), 130.5 (C-15, 15'), 129.9 (C-5, 5'), 124.7 (C-11, 11'), 124.2 (C-7, 7'), 160.9 (C-6,

6'), 37.7 (C-2, 2'), 35.7 (C-1, 1'), 34.3 (C-3, 3'), 27.7 (C-16, 16'), 27.7 (C-17, 17'), 13.7 (C-18, 18'), 12.7 (C-20, 20'), 12.5 (C-19, 19').

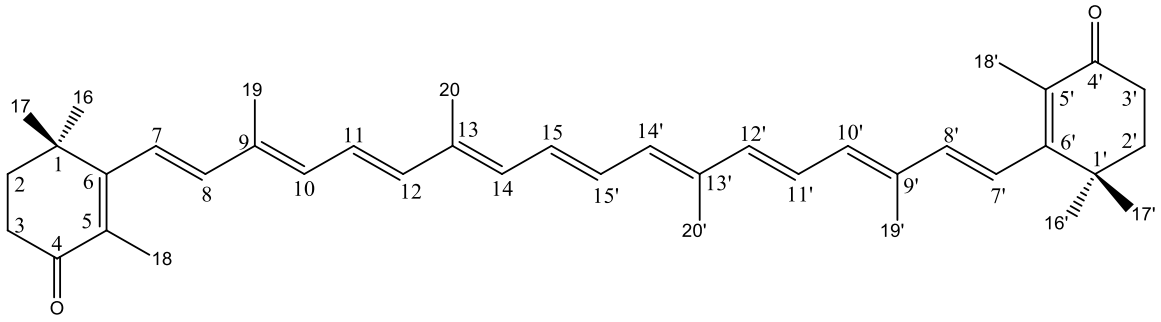

Figure 2. Structure of canthaxanthin

CONCLUSION

In this study, post-fermentation saline bacteria strain *Paracoccus carotinifaciens* VTP20181 was used in a process to extract and isolate canthaxanthin. Chromatography methods were employed to purify the canthaxanthin. Current results suggest new research direction in creating canthaxanthin products from microbiological sources for the food and pharmaceutical industries in Vietnam.

Acknowledgments: This research is funded by project coded No. 10.17/CNSHCB under the project of Biotechnology Development and Application in the Manufacturing Industry to 2020 of the Ministry of Industry and Trade.

REFERENCES

[1] Namitha, K. K., and Negi, P. S., 2010. Chemistry and biotechnology of carotenoids. *Critical Reviews in Food Science and Nutrition*, *50*(8), 728–760. https://doi.org/10.1080/10408398.2010.499811.

[2] Torrissen, O. J., and Christiansen, R., 1995. Requirements for carotenoids in fish diets. *Journal of Applied Ichthyology*, *11*(3–4), 225–230. https://doi.org/10.1111/j.1439-0426.1995.tb00022.x.

[3] Stahl, W., and Sies, H., 2005. Bioactivity and protective effects of natural carotenoids. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1740*(2), 101–107. https://doi.org/10.1016/j.bbadis.2004.12.006.

[4] Liñán-Cabello, M. A., Paniagua-Michel, J., and Hopkins, P. M., 2002. Bioactive roles of carotenoids and retinoids in crustaceans. *Aquaculture Nutrition*, *8*(4), 299–309. https://doi.org/10.1046/j.1365-2095.2002.00221.x.

[5] Liau, B. C., Shen, C. T., Liang, F. P., Hong, S. E., Hsu, S. L., Jong, T. T., and Chang, C. M. J., 2010. Supercritical fluids extraction and anti-solvent purification of carotenoids from microalgae and associated bioactivity. *The Journal of Supercritical Fluids*, *55*(1), 169–175. https://doi.org/10.1016/j.supflu.2010.07.002.

[6] Peng, J., Yuan, J. P., Wu, C. F., and Wang, J. H., 2011. Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: metabolism and bioactivities relevant to human health. *Marine Drugs*, *9*(10), 1806–1828. https://doi.org/10.3390/md9101806.

[7] Novoveská, L., Ross, M. E., Stanley, M. S., Pradelles, R., Wasiolek, V., and Sassi, J. F., 2019. Microalgal carotenoids: A review of production, current markets, regulations, and future direction. *Marine Drugs*, *17*(11), 640. https://doi.org/10.3390/md17110640.

[8] Palozza, P., Maggiano, N., Calviello, G., Lanza, P., Piccioni, E., Ranelletti, F. O., and Bartoli, G. M., 1998. Canthaxanthin induces apoptosis in human cancer cell lines. *Carcinogenesis*, *19*(2), 373–376. https://doi.org/10.1093/carcin/19.2.373.

[9] Veiga-Crespo, P., Blasco, L., Rosa dos Santos, F., Poza, M., and Villa, T. G., 2005. Influence of culture conditions of Gordonia jacobaea MV-26 on canthaxanthin production. *International Microbiology*, *8*(1), 55–58.

[10] Choubert, G., and Storebakken, T., 1989. Dose response to astaxanthin and canthaxanthin pigmentation of rainbow trout fed various dietary carotenoid concentrations. *Aquaculture*, *81*(1), 69–77.

[11] Suhonen, R., and Plosila, M., 1981. The Effect of Beta-Carotene in Combination with Canthaxanthin, Ro 8–8427 (Phenoro®), in Treatment of Polymorphous Light Eruptions. *Dermatology*, *163*(2), 172–176. Doi:10.1159/000250156.

[12] Arden, G. B., Oluwole, J. O. A., Polkinghorne, P., Bird, A. C., Barker, F. M., Norris, P. G., and Hawk, J. L. M., 1989. Monitoring of patients taking canthaxanthin and carotene: an electroretinographic and ophthalmological survey. *Human Toxicology*, *8*(6), 439–450. https://doi.org/10.1177/096032718900800603.

[13] Bhosale, P., and Bernstein, P. S., 2005. Microbial xanthophylls. *Applied Microbiology and Biotechnology*, *68*(4), 445–455. https://doi.org/10.1007/s00253-005-0032-8.

[14] Khodaiyan, K., Faramarz, F., Razavi, S. H., Emam-Djomeh, Z., Mousavi, S. M. A., and Hejazi, M. A., 2007. Effect of culture conditions on canthaxanthin production by Dietzia natronolimnaea HS-1. *Journal of Microbiology and Biotechnology*, *17*(2), 195–201.