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Co-Production of Polyhydroxyalkanoates and Carotenoids by *Haloferax mediterranei* DSM 1411 †

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Abstract: Polyhydroxyalkanoates (PHA) are naturally occurring biopolymers that possess high performance material properties such as biodegradability and biocompatibility. PHA can be produced from renewable carbon sources. However, the industrial production of PHA is still hindered by the costly feed materials. Co-production of other high-value products in addition to PHA can be helpful in alleviating overall production of PHA. In this work, the effect of temperature on PHA and carotenoids co-production by *Haloferax mediterranei* DSM 1411 was investigated using 1% glucose as carbon source. Under batch fermentation at 37 °C, *Haloferax mediterranei* synthesized 3.37 g L^{-1} PHA with concomitant production of 0.76 mg L^{-1} of carotenoids at 144 h. The maximum dry cell weight (DCW) was 6.54 g L^{-1} and PHA content was 51.6%, with 3-hydroxyvalerate (3HV) fraction of 8.01 mol%. By increasing temperature to 42 °C, an increase in PHA and carotenoids production was noticed reaching a maximum of 3.99 g L^{-1} and 0.92 mg L^{-1}, respectively, at 120 h. Likewise, DCW was increased to 7.06 g L^{-1} and PHA content was 56.5%, with 3HV fraction of 8.42 mol%.

Keywords: Polyhydroxyalkanoates; Carotenoids; *Haloferax mediterranei*

1. Introduction

Petrochemical-based plastics are widely used because of their good mechanical and thermal properties, constancy, and durability. However, the accumulation of these nonbiodegradable plastics in the landfills may have negative health and environmental impacts. Recently, many research has focused on the development and production of microbial-derived biodegradable polymers due to their low toxicity and high sustainability that will help to reduce plastic waste accumulation in the environment [1]. Among bioplastics, microbial polyhydroxyalkanoates (PHA), a class of bio-based polymer with properties very similar to synthetic plastics, have attracted research and commercial interests worldwide because of their renewability, biocompatibility, and complete biodegradability [2].

The two most investigated types of PHA are poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). PHB is difficult to process into commodity goods because it is stiff, brittle, has a high degree of crystallinity, and its melting temperature is close to the decomposition temperature. The stoichiometric equation of PHB from glucose gives $C_6H_{12}O_6 + 1.5O_2 \rightarrow C_4H_6O_2 + 2CO_2 + 3H_2O$. The introduction of 3-hydroxyvalerate (3HV) units to form PHBV disrupts the highly crystalline PHB structure, resulting in a polymer with enhanced mechanical properties, quicker degradation rates, and improved physical properties [3].

One of the marine archaea that can produce PHBV through fermentation is *Haloferax mediterranei*. PHBV is accumulated under conditions of excessive carbon content and limited

nutrients (such as nitrogen, phosphorus, dissolved oxygen, and other microcomponents). The monomer ratio of PHBV affects the mechanical and physical properties of biopolymers [4].

The objective of this study was to investigate the effect of temperature on PHA and carotenoids co-production by *Haloferax mediterranei* DSM 1411. Along with PHA, co-production of carotenoids was investigated as an additional high value product to alleviate production costs.

2. Materials and Methods

2.1. Chemicals

All chemicals used in this study were of analytic grade and all solvents were of HPLC grade. Poly(3-hydroxybutyrate), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)] and astaxanthin (Sigma-Aldrich, USA) were used as standards for PHA and carotenoid estimation.

2.2. Microbial Strain

Haloferax mediterranei DSM 1411 was investigated for its ability to co-produce PHA and carotenoids. The selected strain was maintained on *Halobacterium* medium agar Petri dishes and stored at 4 °C for future use.

2.3. Media Preparation

Halobacterium medium consisting of NaCl 156 g L^{-1}, $MgCl_2 \cdot 6H_2O$ 13 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 20 g L^{-1}, $CaCl_2 \cdot 6H_2O$ 1 g L^{-1}, KCl 4 g L^{-1}, $NaHCO_3$ 0.2 g L^{-1}, NaBr 0.5 g L^{-1}, yeast extract 5 g L^{-1}, agar 20 g L^{-1} with glucose 10 g L^{-1} was used for co-production of PHA and carotenoids by *Haloferax mediterranei* DSM 1411. Microbial cultures were prepared by inoculation of single colonies of bacteria in 20 mL of *Halobacterium* broth in separate flasks and incubated at two different temperatures (37 °C and 42 °C) in shaking incubator at 200 rpm.

2.4. Batch Cultivation of Haloferax mediterranei DSM 1411

Batch fermentation was carried out in 500 mL Erlenmeyer flasks containing 200 mL of sterile *Halobacterium* broth medium supplemented with 10 g L^{-1} of glucose. 10% v v^{-1} of microbial culture was then inoculated into the flasks before being incubated at different temperatures (37 °C and 42 °C). Samples were taken every 24 h to measure optical density (OD), dry cell weight (DCW), PHA accumulation, and carotenoids production.

2.5. Analytical Techniques

2.5.1. Optical Density (OD)

Optical density was measured at 600 nm using a UV-visible spectrophotometer (Shimadzu, Japan). 1 mL of culture broth was centrifuged (MiniSpin, Eppendorf) at 13000 rpm for 5 min to separate the supernatant from the pellet. The pellet was then resuspended with 1 mL of washing solution (10% w v^{-1} of NaCl). Optical density was measured using a blank of washing solution for comparison [4].

2.5.2. Dry Cell Weight (DCW)

Dry cell weight was measured to determine the growth of *Haloferax mediterranei* DSM 1411 culture. The culture sample (10 mL) was centrifuged (15,000 rpm, 15 min) and the cell pellet was washed with deionized water after removing the supernatant. The harvested cell pellet was dried at 60 °C until a constant weight was reached and DCW was measured [5].

2.5.3. PHA Quantification

PHA content was determined by acid methanolysis followed by gas chromatography (GC, Agilent 6890A) coupled to a flame ionization detector. Briefly, 1 mL of methanol acidified with 3% (v v^{-1}) H_2SO_4 and 1 mL of chloroform were added to freeze dried cells and the mixture was heated at 100 °C for 3.5 h for depolymerisation and methanolysis of polyesters. Commercially available P(3HB-co-3HV) composed of 88 mol% 3HB and 12 mol% 3HV was used as standard while benzoic acid was used as an internal standard. After cooling, 1 mL of distilled water was added and the suspension was vigorously shaken for 10 min. Two phases were separated thereafter and the organic phase at the bottom layer containing the resulting methylated ester was used for GC analysis. The operating conditions of GC were as follows: injection volume = 1 µL, initial column temperature = 60 °C for 5 min, temperature increase rate = 4 °C min^{-1}, final column temperature = 180 °C for 5 min, carrier gas flow rate = 20 mL min^{-1}, temperature of injection port = 230 °C, and temperature of detection port = 280 °C [1].

2.5.4. Carotenoids Quantification

To determine total carotenoids content, sample cultures were washed with physiological saline solution (0.85% w v^{-1} NaCl) to remove all impurities. Cell pellets were then mixed with 5 mL of acetone, briefly sonicated, and incubated for 15 min at 55 °C [6,7] for carotenoids extraction and the suspension was centrifuged at 10,000 rpm for 15 min. The absorbance of the supernatant was then measured at 476 nm using a spectrophotometer (Shimadzu, Japan).

3. Results and Discussion

Optical density was measured to monitor biomass formation in the culture medium. The change in optical density at different temperatures is shown in Figure 1. A maximum OD value of 21.8 was noticed at 120 h for *Haloferax mediterranei* culture incubated at 42 °C. This value was 26% higher than that recorded for *Haloferax mediterranei* culture incubated at 37 °C which indicates the positive effect of temperature on the growth of *Haloferax mediterranei* cells and accumulation of PHA as a growth-associated product [5].

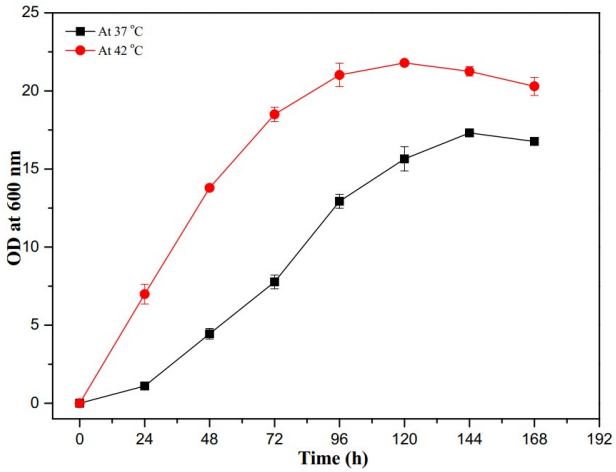

Figure 1. Effect of temperature on the growth of *Haloferax mediterranei* DSM 1411 using glucose as carbon source.

Time profile of DCW, PHA, 3HV, and carotenoids production by *Haloferax mediterranei* DSM 1411 using glucose as carbon source at 37 °C is shown in Figure 2. A gradual increase in cell growth of *Haloferax mediterranei* DSM 1411 demonstrates the ability of the investigated microorganism to utilize glucose. A maximum DCW of 6.54 g L^{-1} was obtained at 144 h of cultivation with PHA accumulation of 3.37 g L^{-1} (51.6% of DCW), 3HV fraction of 8.01 mol%, and carotenoids production

of 0.76 mg L^{-1}. PHA production in this study was higher than that obtained previously by Alsafadi and Al-Mashaqbeh [8] who reported a maximum PHA production of 43% (as a percentage of DCW) with a 3HV fraction of 6.5 mol% when cultivating *Haloferax mediterranei* DSM 1411 in nutrient-rich AS-168 medium supplemented with olive mill wastewater. This finding demonstrates the potential use of glucose as carbon source for bioplastics production.

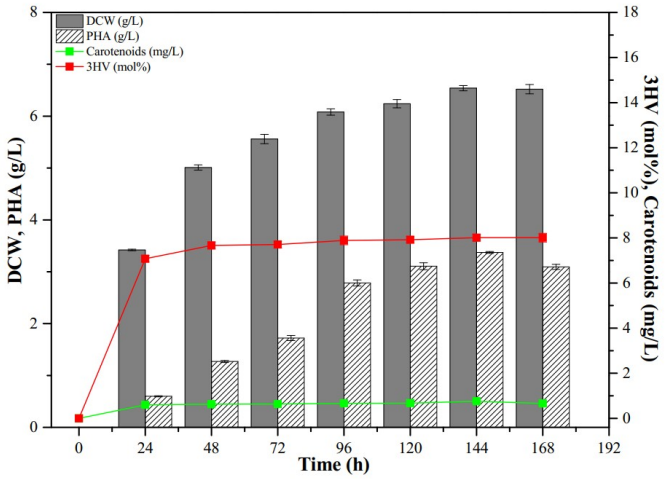

Figure 2. Time profile of DCW, PHA, 3HV, and carotenoids production by *Haloferax mediterranei* DSM 1411 cultivated at 37 °C using glucose as carbon source. Data are average values ± standard deviation of triplicate experiments.

As shown in Figure 3, increasing temperature from 37 °C to 42 °C led to an increase in both PHA and carotenoids production reaching a maximum of 3.99 g L^{-1} and 0.92 mg L^{-1}, respectively, at 120 h. Likewise, DCW was increased to 7.06 g L^{-1} and PHA content was 56.5%, with a 3HV fraction of 8.42 mol%. PHA and carotenoids accumulation in this study was higher than that obtained previously by Kumar and Kim [9] who reported a PHA and carotenoids production of 1.0 g L^{-1} and 0.89 mg L^{-1} upon upscaling biotransformation of waste cooking oil to 1 L fermenter by *Paracoccus* sp. LL1. Likewise, our results were higher that those obtained by Ghosh et al. [3] who reported a PHA concentration of 2.2 g L^{-1} and PHBV (3 mol% 3HV) when using 25% (w w^{-1}) of Ulva sp. hydrolysate as a carbon source by *Haloferax mediterranei* cultivated at 42 °C.

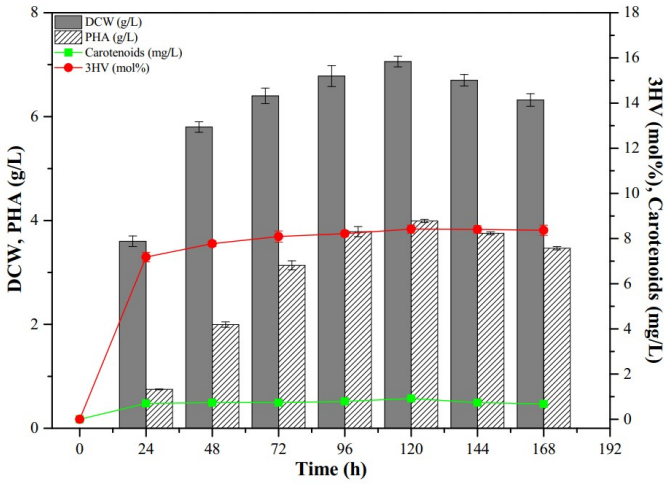

Figure 3. Time profile of DCW, PHA, 3HV and carotenoids production by *Haloferax mediterranei* DSM 1411 cultivated at 42 °C using glucose as carbon source. Data are average values ± standard deviation of triplicate experiments.

4. Conclusions

This study provides a promising strategy for the co-production of PHA and carotenoids by *Haloferax mediterranei* DSM 1411 which was able to convert glucose into carotenoids and PHA. Increasing temperature from 37 °C to 42 °C led to an increase in both PHA and carotenoids production, reaching a maximum of 3.99 g L^{-1} and 0.92 mg L^{-1}, respectively, at 120 h. *Haloferax mediterranei* DSM 1411 cultivated at 42 °C holds the potential for co-producing value added bioproducts. However, further improvement in the fermentation strategies including the use of co-substrates and metabolic flux balance is required to achieve higher yields for both PHA and carotenoids.

Author Contributions: C.K. and B.S.K. conceived and designed the experiments; C.K. performed the experiments and analyzed the data; C.K. wrote original draft paper; B.S.K. reviewed and edited the paper. All authors have read and agreed to the published version of the manuscript

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

PHA	poylhydroxyalanoates
PHB or 3HB	poly(3-hydroxybutyrate)
3HV	poly(3- hydroxyvalerate)
PHBV	poly(3-hydroxybutyrate-co-3-hydroxyvalerate)

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