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Short communication

Improved production of lycopene and β-carotene by *Blakeslea trispora* with oxygen-vectors

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Abstract

Lycopene and β-carotene production were increased when oxygen-vectors, *n*-hexane and *n*-dodecane, were added to cultures of *Blakeslea trispora* because of the enhanced dissolved oxygen concentrations. With 1% (v/v) *n*-hexane or *n*-dodecane added in the medium, lycopene production was 51% or 78% higher and β-carotene production was 44% or 65% higher than that of the control, respectively. The highest lycopene and β-carotene production, 533 mg $l^{-1}$and 596 mg l^{-1}, were obtained when 1% (v/v) *n*-dodecane and 0.1% (w/v) Span 20 were added together, which were 2.1-fold and 1.8-fold of the control, respectively.

Keywords: Lycopene; β-Carotene; *Blakeslea trispora*; Oxygen-vectors; Span 20

1. Introduction

Lycopene and β-carotene, two kinds of important fat soluble carotenoids, are essential nutrients in human diet because they prevent cardiovascular diseases, regulate the immuno system and are considered as anti-carcinogenic agents and antioxidants [1,2]. The classical, natural sources of carotenoids are fruits, vegetables and microorganisms. The zygomycete, *Blakeslea trispora*, is used on an industrial scale to produce β-carotene [3,4], and a semi-industrial process has also been developed for lycopene production [5].

B. trispora is an aerobic microorganism and sufficient supply of oxygen can improve both cell growth and carotenoid synthesis [6,7]. However, the high viscosity of the fermentation broth, the intertwined mycelial growth of *B. trispora* and the low solubility of oxygen in water result in a deficiency of the dissolved oxygen in the fermentation medium [8,9]. How to increase the concentration of dissolved oxygen in the medium is very important for the culture of *B. trispora*. One approach to achieve better oxygen supply is by the addition of oxygen-vectors, which can increase the apparent solubility of oxygen in the medium [10,11]. Oxygen-vectors are hydrophobic liquids in which oxygen has a higher solubility than in water. Hemoglobin, perfluorochemicals and hydrocarbons are generally used as oxygen-vectors in biotechnology [12,13]. Hydrocarbons are more favored for large-scale fermentation because they are cheaper compared to hemoglobin and perfluorochemicals. In this paper, the effects of two oxygen-vectors, *n*-hexane and *n*-dodecane, on the production of lycopene and β-carotene by *B. trispora* have been investigated in order to enhance the fermentation efficiency.

2. Material and methods

2.1. Microorganism and culture medium

B. trispora ATCC 14271, mating type (+), and ATCC 14272, mating type (−), which were maintained on potato dextrose agar slants, were grown in the seed medium (starch 40 g l^{-1}, corn hydrolysate 50 g l^{-1}, KH_2PO_4 1 g l^{-1}, $MgSO_4$ 0.1 g l^{-1}, Vitamin B_1 0.01 g l^{-1}, pH 6.5) at 28 °C for 40 h in 500 ml flasks containing 100 ml medium. The cultures were used for the inoculation of the fermentation medium.

2.2. Fermentation conditions

The fermentation was carried out in 500 ml flasks with 100 ml medium including 10% inoculum, and the inoculum was a 1:2 (volume ratio) mixture of ATCC 14271 (+) and ATCC 14272 (−). The fermentation medium had the following composition (g l^{-1}): starch 40; soybean meal 20; corn hydrolysate 25; KH_2PO_4 1; $MgSO_4$ 0.1; Vitamin B_1 0.01; pH 6.5. Before autoclaving the components of the medium were heated until dissolved completely.

In addition to the control, sterilized *n*-hexane or *n*-dodecane was added to the fermentation medium at the concentration of 1% (v/v) and Span 20 at 0.1% (w/v) or as otherwise stated. The flasks were incubated at 28 °C in a rotary

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Table 1
Dry cell weight, lycopene production and relative dissolved oxygen concentration of *Blakeslea trispora* with different concentrations of oxygen-vectors added at 0 day

Concentration of oxygen-vectors (%, v/v)	Dry cell weight ($g\ l^{-1}$)	Lycopene content ($mg\ g^{-1}$)	Lycopene production ($mg\ l^{-1}$)	Relative dissolved oxygen concentration (%)
Control	53 ± 3	4.6 ± 0.4	245 ± 9	100
n-Hexane				
0.3	53 ± 2	5.5 ± 0.2	294 ± 9	102.3
0.5	54 ± 4	5.8 ± 0.5	310 ± 20	105.6
1	55 ± 4	6.8 ± 0.3	371 ± 14	109.7
1.5	55 ± 5	5.7 ± 0.7	309 ± 16	109.1
2.5	55 ± 9	2.3 ± 0.3	129 ± 11	107.0
n-Dodecane				
0.3	54 ± 1	6.1 ± 1.0	326 ± 11	111.6
0.5	60 ± 7	5.8 ± 0.5	346 ± 15	116.7
1	60 ± 4	7.3 ± 0.8	437 ± 10	122.7
1.5	60 ± 8	6.1 ± 0.3	366 ± 20	122.1
2.5	61 ± 11	6.2 ± 0.3	377 ± 12	120.3

The cells were harvested on the 5th day and 5 $mmol\ l^{-1}$ nicotine was added on the 2nd day. Values are means of triplicate ± standard deviation.

shaker incubator and the inhibitor, 5 $mmol\ l^{-1}$ nicotine, was added on the 2nd day for the lycopene production. Cultures were maintained for 5 days, and then the dry cell weight, lycopene and β-carotene contents were determined. All culture experiments and estimations were carried out in triplicate.

2.3. Analytical methods

The cell mass was filtered through muslin, washed thoroughly with distilled water, and dried in a vacuum drier at 45 °C under 0.08 MPa vacuum for 48 h. The dried cells were weighed to determine cell growth. The relative dissolved oxygen concentration of the culture medium was determined with a dissolved oxygen meter (DO-28 model, China). To extract lycopene or β-carotene, the dried cells were cut into small pieces and homogenized in petroleum ether and shaken until they became colorless. The petroleum ether phase containing lycopene or β-carotene was analyzed by a high performance liquid chromatograph (HPLC) equipped with a Diamonsil C_{18} column (250 mm × 4.6 mm) at 28 °C and acetonitrile:dichloromethane (75:25, v/v) as the mobile phase at 1.5 $ml\ min^{-1}$. The absorption of the carotenoids was measured at 450 nm. Lycopene or β-carotene was identified by comparing with the retention time of the standard sample, and quantitative analysis was performed by the single-point calibration method using an external standard (95% lycopene or 96% β-carotene).

3. Results and discussion

3.1. Effects of concentrations of oxygen-vectors on lycopene and β-carotene production

The advantage of using oxygen-vectors in fermentation was that it could increase the oxygen transfer rate from the gas phase to the microorganisms without the need for extra energy supply. The concentrations of oxygen-vectors have some influence on oxygen solubility. With 1% (v/v) *n*-hexane or *n*-dodecane the dissolved oxygen concentration of the medium reached the maximum value and then decreased slightly with a further increase in the oxygen-vectors (Tables 1 and 2). The results were consistent with experiments previously reported [11,13] on the rheological behavior of this emulsion and the reason may be that in the broken slope region, there is a notable increase in the apparent viscosity, which might affect the oxygen transfer rates. The amount of *n*-hexane or *n*-dodecane in the medium

Table 2
Dry cell weight, β-carotene production and relative dissolved oxygen concentration of *B. trispora* with different concentrations of oxygen-vectors added at 0 day

Concentration of oxygen-vectors (%, v/v)	Dry cell weight ($g\ l^{-1}$)	β-Carotene content ($mg\ g^{-1}$)	β-Carotene production ($mg\ l^{-1}$)	Relative dissolved oxygen concentration (%)
Control	55 ± 5	6.2 ± 0.6	341 ± 11	100
n-Hexane				
0.3	55 ± 6	7.1 ± 0.7	391 ± 8	103.1
0.5	57 ± 5	7.3 ± 0.9	416 ± 15	106.7
1	57 ± 8	8.6 ± 0.6	490 ± 10	110.5
1.5	58 ± 5	8.1 ± 0.7	469 ± 13	109.4
2.5	57 ± 4	5.6 ± 0.5	319 ± 11	106.3
n-Dodecane				
0.3	56 ± 8	7.8 ± 0.6	436 ± 10	112.1
0.5	61 ± 4	7.9 ± 0.9	482 ± 16	117.4
1	61 ± 9	9.2 ± 1.1	561 ± 21	124.1
1.5	63 ± 10	8.5 ± 0.6	535 ± 13	122.9
2.5	61 ± 13	8.1 ± 0.8	494 ± 19	120.1

The cells were harvested on the 5th day. Values are means of triplicate ± standard deviation.