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Effect of Oxidative Stress on *Kocuria marina* DAGII during β-cryptoxanthin Biosynthesis

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Abstract—*During fermentation, aerobic metabolism generates reactive oxygen species (ROS) such as hydrogen peroxide, superoxide radicals. These species are highly damaging to cellular constituents, thereby resulting in a condition of oxidative stress. Oxidative stress is considered as a disturbance in pro-oxidant and antioxidant balance which causes potential cell damage. Various researchers have reported oxidative stress induced carotenoid production in various microorganisms. In the present study, effect of oxidative stress induced by H_2O_2 on carotenoid production by Kocuria marina DAGII has been investigated. H_2O_2 has significant advantages over other compound (like iron ions, BHT, liquid paraffin) induced oxidative stress due to its low cost, non-environmental pollution and suitability for mass production. Kocuria marina DAGII is a gram positive aerobic β-cryptoxanthin producing bacterium. Flasks containing Glucose (7.5 g/L), Maltose (10 g/L), Yeast Extract (10 g/L), Peptone (5 g/L) and NaCl (4 g/L) were supplemented with H_2O_2 in increasing concentration (0, 20, 40, 60, 80, 100, 120, 140 and 200 µM) and then inoculated with 1% inoculum of Kocuria marina DAGII. Fermentation was conducted at 25°C, 120 rpm for 3 days. After 2 days, dry biomass significantly increased from 9.8 to 11.95 g/L when the H_2O_2 concentration increased from 0 to 60 µM, but remarkably decreased from 11.95 to 8.5 g/L when H_2O_2 concentration increased from 60 to 200 µM. This indicated that addition of H_2O_2 at higher concentration was toxic to cell thereby causing cellular damage. In addition, the production of β-cryptoxanthin increased from 3.3 to 3.86 mg/L with the increase in H_2O_2 concentration from 0 to 60 µM. However, the yield decreased to 3.26 mg/L when the H_2O_2 concentration increased from 60 to 200 µM. Overall, low concentrations of H_2O_2 increased the production of biomass and β-cryptoxanthin whereas high concentrations of H_2O_2 (above 60 µM) decreased the production of biomass and β-cryptoxanthin.*

1. INTRODUCTION

β-cryptoxanthin (β-CRX) is a hydroxylated β-carotene having multiple functions including antioxidant and anti-cancer activities, promotion of bone health and improvement of the immune function [1]. It is the only xanthophyll possessing pro-vitamin A activity [2]. Citrus fruits such as tangerines, oranges, mandarins are the primary natural sources of β-CRX. However, it is also widely distributed in red paprika, leafy vegetables, pumpkins, persimmons and some yellow-coloured animal products such as egg yolk and butter [3]. In last few decades, emphasis has been laid on microbial production of carotenoids due to its ability to fulfil increasing demands of natural carotenoids [4]. A wide variety of carotenoid producing microbes have been already reported [5]. However, microbial β-CRX is limited due to unavailability of suitable microbes and fermentation conditions. Few microbes like *Flavobacterium lutescens*, *Brevibacterium linens* and *Flavobacterium multivorum* have been reported as β-CRX producers.

Oxidative stress is essentially considered as an imbalance between pro-oxidant and antioxidant that causes potential cell damage. Formation of reactive oxygen species (ROS) such as hydrogen peroxide, hydrogen and superoxide radicals during respiration might cause oxidative injury to cells, thereby, leading to the situation of oxidative stress [6]. Cells use their enzymatic and non-enzymatic defense systems such as carotenoids in order to counteract these damages [6]. Thus, it might be possible that carotenoid synthesis would be affected by oxidative stress [7].

Kocuria marina DAGII is a gram positive, yellow colored, aerobic cocci that was previously isolated in our laboratory during routine screening of pigment producing bacterium. It was further confirmed to be a β-CRX producer. The objective of this study was to examine the effect of oxidative stress, induced by H_2O_2, on β-CRX production by *Kocuria marina* DAGII. H_2O_2 was preferred over other compounds like iron ions, BHT, liquid paraffin because of its low cost, ease of availability, non-environmental pollution and suitability for mass production. The growth and β-CRX yield at varying H_2O_2 concentrations were studied in order to investigate the response of *Kocuria marina* DAGII induced by H_2O_2 during β-CRX production.

2. MATERIALS AND METHODS

2.1. Microorganism

Kocuria marina DAGII, a yellow colored gram positive β-CRX producing bacterium was isolated in our laboratory during routine screening of pigment producing bacterium. The bacterium was maintained on Brain Heart Infusion Agar (BHIA) at 4°C and subcultured every month..

2.2. Fermentation conditions

The strain was grown in 50mL of cultivation medium containing glucose (7.5 g/L), maltose (10 g/L), yeast extract (10 g/L), bacteriological peptone (5 g/L) and NaCl (4 g/L). The initial pH was adjusted to 7.9. Fermentation was done at 25°C on a rotary shaker (150 rpm) (New Brunswick Innova® 42, NJ, USA) for 3 days. The flasks were inoculated with 1% v/v of culture having an optical density of 0.4-0.6. For studying the effect of H_2O_2, the cultivation medium was supplemented with H_2O_2 of different concentrations (0, 20, 40, 60, 80, 100, 120, 140 and 200 µM).

2.3. β-CRX extraction

Bacterial cultures were analysed for biomass and β-CRX concentrations according to the methods of Mitra et al. (2015) [1]. Briefly, biomass was assessed by dry biomass method and the pigment was extracted by two-stage extraction method. Extracted pigment in methanol was transferred to petroleum benzene and analysed by scanning the absorbance in the wavelength range of 360-650nm. The maximum absorbance was observed at 445nm (λmax) and the pigment concentrations were determined through a standard curve prepared by using commercial β-CRX.

2.4. Statistical analysis

All the experiments were conducted in triplicates. The values represented are the mean ± SD of three replicates which was done using Microsoft Excel 2010.

3. RESULTS AND DISCUSSION

3.1. Effect of different concentrations of H_2O_2 on biomass production

The effect of H_2O_2 on β-CRX production were examined after adding different concentrations of H_2O_2 to the cultivation medium inoculated with 1%(v/v) of bacterial culture. After 2 days of culturing, dry biomass increased significantly from 9.8 to 11.95 g/L, when the H_2O_2 concentration increased from 0 to 60 µM (Fig. 1).

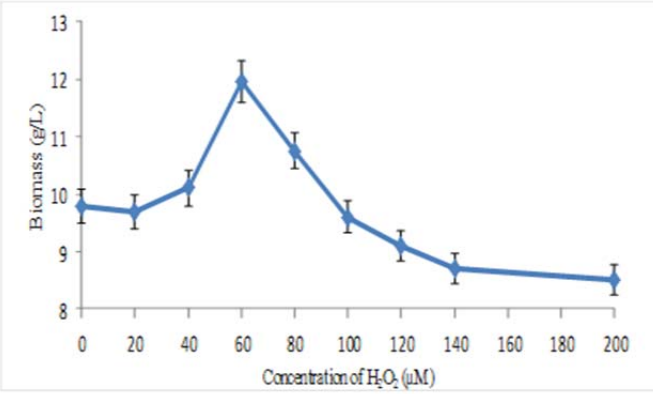

Fig. 1: Effect of H_2O_2 on biomass production

But on increasing the H_2O_2 concentration from 60 to 200 µM, the dry biomass remarkably decreased from 11.95 to 8.5 g/L (Fig. 1). This phenomenon probably indicated that addition of H_2O_2 above 60 µM led to oxidative stress which resulted in reduced biomass production.

3.2. Effect of different concentrations of H_2O_2 on β-CRX production

Fig. 2 showed the effect of H_2O_2 on β-CRX production by *Kocuria marina* DAGII. The production of β-CRX increased from 3.3 to 3.86 mg/L with increase in the added amount of H_2O_2 concentration from 0 to 60 µM (Fig. 2). After 60 µM, the β-CRX production decreased from 3.86 to 3.26 mg/L (Fig. 2). Thus, β-CRX yield could be increased by mild oxidative stress, i.e., low concentrations of H_2O_2. However, at high concentrations of H_2O_2, it was probable that part of β-CRX produced was getting consumed as antioxidant, thereby reducing its production yield [8]. A similar study reported the enhancement of β-carotene production when *Blakeslea trispora* was treated with 10 µM of H_2O_2 [7].

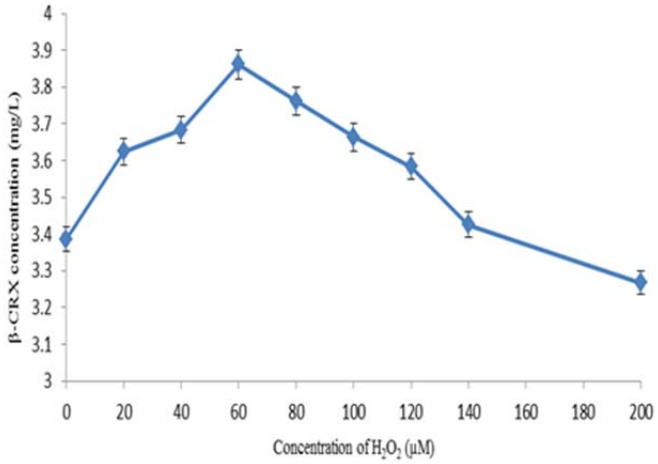

Fig. 2: Effect of H_2O_2 on β-CRX production

4. CONCLUSION

β-CRX is a bioactive compound having huge implications due its therapeutical and nutraceutical aspects. Response of *Kocuria marina* DAGII, a natural β-CRX producer has been studied under conditions of oxidative stress. The results indicated that production of β-CRX and biomass can be improved by mild oxidative stress (60 µM H_2O_2). H_2O_2 concentrations above 60 µM were found to have inhibitory effect on cell growth and β-CRX production. However, the reasons for the enhancement of β-CRX are yet to be determined.

5. ACKNOWLEDGEMENT

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