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Production of violacein pigment using solid state fermentation

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GRAPHICAL ABSTRACT

Violacein extracted from sugarcane bagasse in ethyl acetate

ABSTRACT

Natural pigment from *Chromobacterium violaceum*, violacein is highly in demand due to its pharmacological properties. Submerged fermentation of *Chromobacterium violaceum* results in low yield of violacein due to extraction problems. Hence, solid state fermentation is proposed where separation of pigments is easier. Solid state fermentation of *Chromobacterium violaceum* UTM5 on sugarcane bagasse was tested in shake flask and a column system. Crude violacein was extracted using ethyl acetate and characterized using UV-Visible Spectrophotometer and Fourier Transform Spectroscopy (FTIR). Highest violacein production (5.11 mg/g SCB) was observed in sugarcane bagasse supplemented with 100 mg/L of L-tryptophan at 30 °C for three days. The violacein observed at 556 nm using UV-Vis spectra and within the range of λ_{max} as reported by other researchers. FTIR spectrum of violacein showed a weak band of OH stretching at 3215.98 cm^{-1} which might overlap with the N-H band. Two stretching bands at 1603.98 cm^{-1} and 1702.85 cm^{-1} were assigned to the C=O amide groups. Intermediate C-N band was observed at 1203.62 cm^{-1}. This study showed that the violacein produced in solid state fermentation was quite similar as that produced using submerged fermentation.

Keywords: Solid state fermentation, Chromobacterium violaceum, violacein, sugarcane bagasse, natural pigment

1. INTRODUCTION

The advantages of pigment production from microorganisms include easy and fast growth in the cheap culture medium, independence from weather conditions and colors of different shades [1]. Nowadays, there has been an increasing trend towards replacement of synthetic colorants by natural pigments because of the high consumer demand for natural products. Consumers have realized that natural products are much safer and environmental friendly [2].

Advancements in fermentation techniques have led to the easy production and isolation of colour pigments. Microbial pigments can be produced either by solid state fermentation or submerged fermentation. Submerged fermentation has been used for the growth of *Chromobacterium violaceum* UTM5. However, separation of violacein from submerged fermentation involves many steps and has low recovery and low yield. The use of solid state fermentation will facilitate the recovery of pigment using a single step, hence facilitating the recovery of pigments by offering higher end-concentration of products, higher fermentation productivity, lower cost of down-stream processing and lower demand on sterility due to the low water activity used in solid state fermentation [3]. Due to high cost of synthetic medium, there is a need to develop low cost process and extraction procedure for the production of pigments using waste organic materials for large scale production of microbial pigments. Hence, sugarcane bagasse have been used as the low cost media in the production of violacein pigment.

Violacein is a versatile pigment from a bacterium *Chromobacterium violaceum* that exhibits several biological activities. It has gained increasing importance in industrial markets, such as in medicine, cosmetics, food and textiles [2]. The structure of violacein is as shown in figure 1.

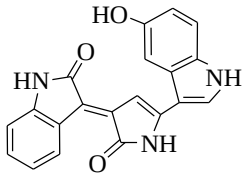

Figure 1 The structure of violacein

This research will emphasize on growing *Chromobacterium violaceum* UTM5 in sugarcane bagasse (SCB), extract the violacein grown in solid state fermentation (SSF) using various solvents and also characterize the violacein obtained from solid state fermentation (SSF) using UV-Visible Spectrophotometer and Fourier Transform Infrared Spectroscopy (FTIR).

2. EXPERIMENTAL

The experiment was divided into three main phases. The first phase focused on growing the *Chromobacterium violaceum* on the sugarcane bagasse in the shake flask then continued in the column system. Then, the second phase was the extraction of violacein pigment grown on sugarcane bagasse with ethyl acetate. The last step was the characterization violacein using instrumental analysis such as UV-Visible spectroscopy and FTIR. The figure 2 show the flow chart of the present study.

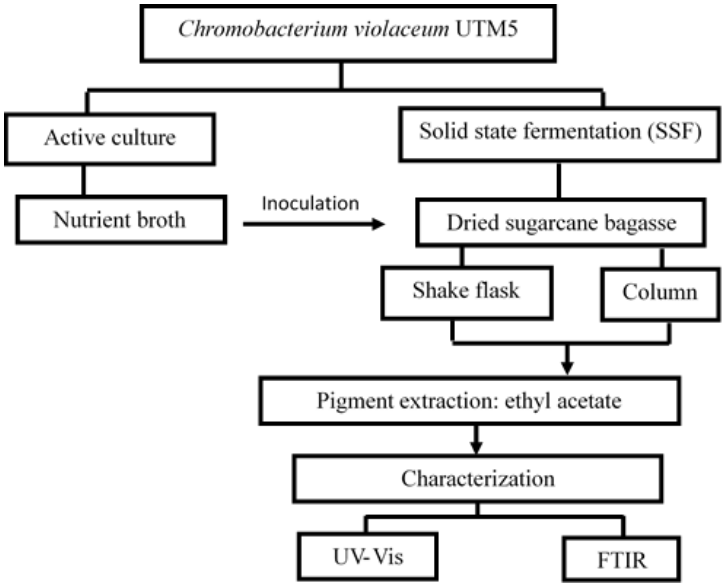

Figure 2 The flow chart of the present study

3. RESULTS AND DISCUSSION

3.1. Production of Violacein in Shake Flask

It was observed that sugarcane bagasse is able to support the growth *C. violaceum* UTM5. The colorless SCB changed to purplish after three days of incubation However, the percentage yield of violacein are negligible and has poor pigment production on sugarcane bagasse inside the shake flask.

However, lower pigment production could be observed in the shake flask due to poor aeration. Poor aeration promotes anaerobic condition which *C. violaceum* is able to live under anaerobic and aerobic conditions but it produced only in the presence of oxygen [4]. Growth of *C. violaceum* UTM5 and violacein production on SCB before and after 3 days shown in figure 3.

Figure 3 Growth of C. violaceum UTM 5 and violacein production on SCB before and after 3 days

3.2 Production of Violacein in Column

Column system provide better growth condition for *C. violaceum* UTM5 and able to produce violacein pigments up to 5.11 mg/g SCB. The temperature of the column was control by the air-conditioner were set up at 30 °C. Since the *C. violaceum* is an aerobic system, the cycle of bacteria in column was able to enhance the production of the bacteria and the attachment of bacteria to the sugarcane bagasse.

Since sugarcane bagasse is an agricultural waste media, it is necessary to supplement it with additional nutrients, namely nitrogen source to increase the pigment production of *C. violaceum* UTM5 as reported by Aruldass *et al*. The yield of pigment significantly increased as the concentration of L-tryptophan used. A maximum yield at 5.11 mg/g SCB was achieved when supplemented 100 mg/L of L-tryptophan. Figure 4 show the growth of *C. violaceum* on SCB in column, (a) absence of L-tryptophan and (b) present of L-tryptophan.

(a) (b)

Figure 4 Growth of C. violaceum on SCB in column, (a) absence of L-tryptophan and (b) present of L-tryptophan

3.3 Characterization of Violacein Pigment

The maximum absorbance for violacein was obtained through UV-Vis analysis. UV-Vis spectra for the violet pigment showed a maximum peak with absorption at 556 nm (Figure 5) due to the presence of chromophore groups (C=C and C=O) which is responsible for electronic absorption. The carbonyl groups absorb intensely at the short wavelength end of the spectrum but carbonyl group has less intense bands at higher wavelength due to the participation of n electrons [5].

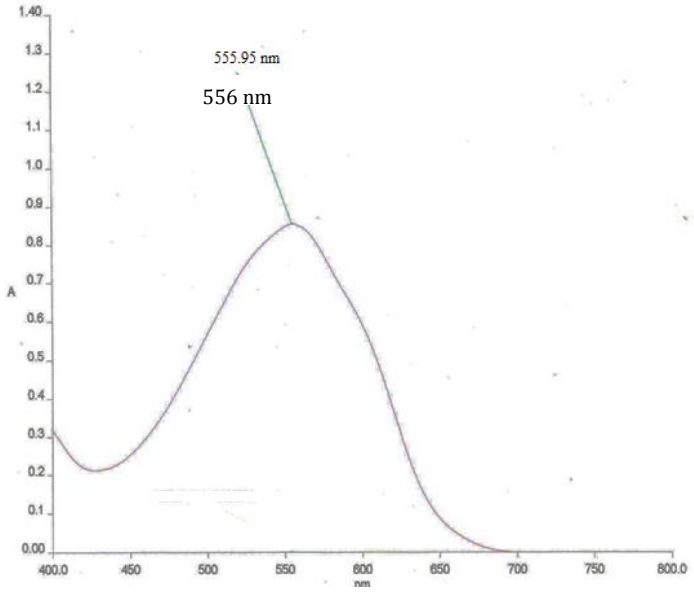

Figure 5 UV-Vis spectra of violacein

FTIR spectrum of violacein showed a weak band of OH stretching at 3215.98 cm^{-1} which might overlap with the

N-H band. Two stretching bands at 1603.98 cm^{-1} and 1702.85 cm^{-1} were assigned to the C=O amide groups. Intermediate C-N band was observed at 1203.62 cm^{-1}. Figure 6 show the FTIR spectra of violacein.

Secondary amides are associated through hydrogen bonding to form dimers of cis configuration and trans configuration resulting in the replacement of free N-H stretching band. The weak band at 3215.98 cm^{-1} may be due to an overtone of the band at 1518.53 cm^{-1} (trans secondary amide) or combination of C=O stretching and N-H in-plane bending (cis secondary amide) [5].

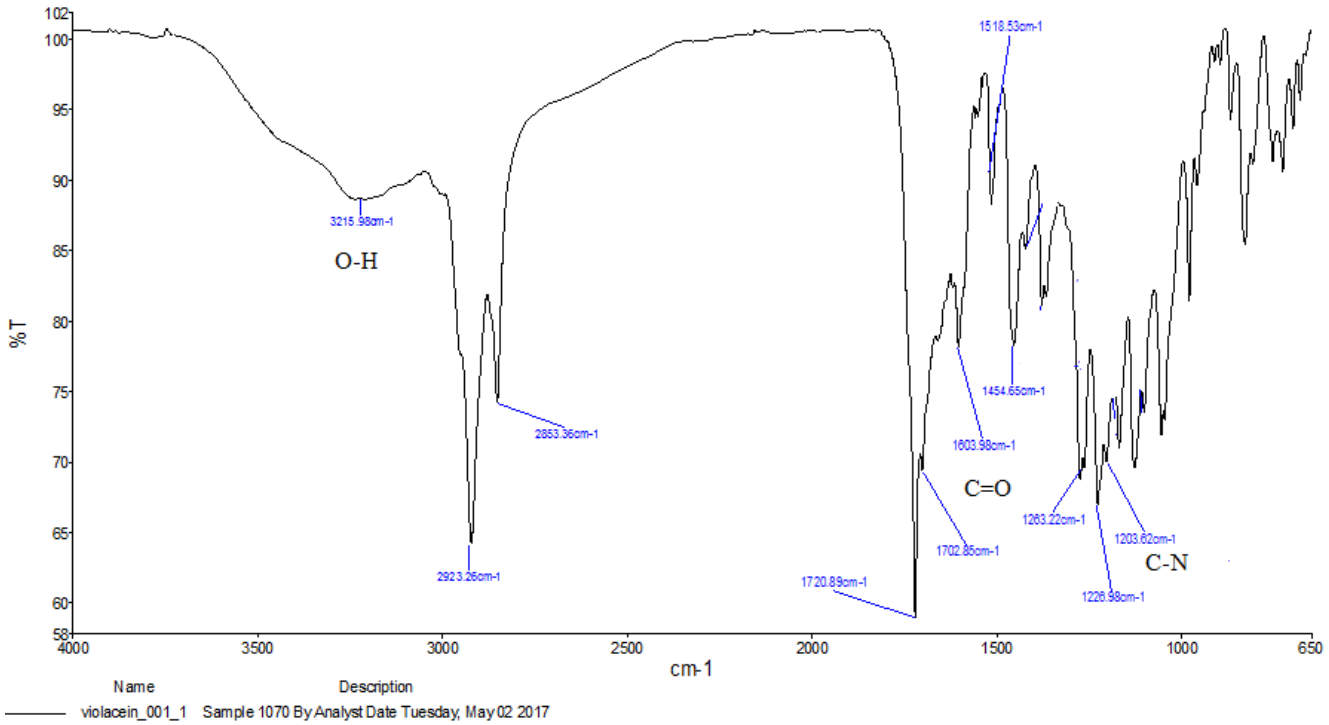

Figure 6 FT-IR spectra of violacein

The C=O stretching of this amide was found as doublet at 1702.85 cm^{-1} and 1603.98 cm^{-1}. This doublet is highly characteristic of amides where the higher frequency band is predominantly C=O (amide I band) and the lower is predominantly N-H in-plane bending (amide II band) [5].

The frequency of C=O group for this compound is lower due to the conjugation of the carbonyl group with an aromatic ring results in the delocalization of the electrons of both unsaturated groups and reduces the double bond character of both the bonds causing a lowering of carbonyl frequency from 1718 cm^{-1} (normal absorption frequency for carbonyl) to 1702.85 cm^{-1}. The lowering of absorption frequencies of C=O groups are attributed to the resonance [5].

4. CONCLUSION

As a conclusion, *Chromobacterium violaceum* was found to be able to grow on sugarcane bagasse in shake flask and column system through solid state fermentation. Highest production of violacein was observed in sugarcane bagasse supplemented with 100 mg/L of L-tryptophan at 30 °C for three days. The extraction of crude violacein was successfully carried out using ethyl acetateand characterized using UV-Visible Spectrophotometer and Fourier Transform Spectroscopy (FTIR). The solid state fermentation method for violacein production quite similar to submerged fermentation and SSF is a better technique as the violacein was easily separated from the bacteria cell and lower the cost of production.

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