Identification and characterization of a newly isolated carotenoid producing bacterium

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Abstract

A reddish coloured colony was isolated during routine screening of pigment producing microorganism in the Department of Biotechnology, NIT Durgapur. Preliminary evaluation revealed the microbe to be a Gram positive Coccus. The bacterium was maintained on Brain Heart Infusion plates. For analytical purposes, shake flask experiments were conducted by sub culturing the bacterium in Brain Heart Infusion broth and then incubating at 30°C and 120 rpm for 120h. The intracellular content of the bacterium was extracted by cell lysis using methanol. The obtained extract in methanol was scanned in a UV-Vis spectrophotometer and it revealed three distinct peaks at 445nm, 466nm and 492nm. The obtained absorption peaks are a key characteristic feature of carotenoid which led us to establish that the pigment might be a carotenoid. The absorption spectrum was obtained in different solvent system like petroleum benzene, hexane and chloroform. The results suggested that the obtained carotenoid could be Lycopene. As an initial study, Lycopene extract was obtained from tomato (Lycopersicon esculentum) and the absorption spectrum of the lycopene extract was compared with the obtained bacterial carotenoid. Extraction of pigment from microbial sources has become a promising field in the research and development. Under this scenario, our findings hold a good prospect in production of microbial carotenoids.

Key words: *Carotenoids, Lycopene, Kocuria sp., 16s rDNA sequencing, Growth profile Absorption spectrum.*

I. INTRODUCTION

Microbes produce pigments mostly as secondary metabolites, which are often harvested using fermentation technology. Carotenoids are one such pigment that is not only present in various fruits and vegetables but is also synthesized by different micro-organisms. Broadly, carotenoids are divided into two major groups, i.e., carotene which consists of hydrocarbon and xanthophylls which consists of oxygenated derivatives of hydrocarbon. Carotenoids play diverse function as photosynthetic pigment, anti-oxidant, vitamin A precursor [1]. Most importantly, the color and biological activity of carotenoids is due to the presence of conjugated double bond in its structure [2,3]. Nowadays carotenoids have gained importance primarily because of their bright yellow to red pigmentation and their beneficial effects on mankind [4]. Numerous observational studies have suggested that carotenoids are preventive against gastric cancer [5]. It has also been suggested that dietary xanthophylls inhibits the onset of arteriosclerosis, cataract, age-related macular degeneration, multiple sclerosis and cancer [6]. Due to these health benefits, carotenoids are being used in various industries like pharmaceutical, nutraceutical, cosmetic industries [4,5,6]. Moreover, consumer's aversion regarding synthetic food additives has increased the acceptability of natural carotenoids in food and feed industries [4]. In recent years, production of carotenoids through fermentation technology has increased [7]. The major reason is attributed to the fact that fermentation is faster and more productive process compared to other chemical processes. Microbes are highly versatile since they can be easily manipulated using genetic engineering and can be easily grown on simple substrates [8]. Moreover unlike other natural sources such as plants, pigment production using microbe is not subjected to seasonal variation as well as weather conditions [9].

Under this context, a reddish colored colony was isolated in our laboratory during routine screening of pigment producing micro-organism. Identification and growth profiling of the bacterium was done. Extraction and preliminary studies on pigment identification suggested that the produced pigment could be carotenoid, i.e., Lycopene.

II. EXPERIMENTAL

A. *Chemicals-*

Brain Heart Infusion was purchased from Hi-media Laboratories, India. Solvents used like Methanol, Petroleum Benzene, Hexane, Chloroform, Ethyl acetate were obtained from Merck, India.

B. *Isolation, identification and culture condition of the microorganism-*

A random soil sample was collected and a strain was isolated from it. The Brain Heart Infusion Agar (BHIA) was used to maintain the isolated strain. The media was autoclaved at 15 psi, 121°C for 15minutes. The strain was regularly subcultured in 250mL Erlenmeyer flask containing 50mL of medium (Brain Heart Infusion). The culture flasks were further incubated at 30°C on a rotary speed of 120rpm for 120h.

The identification of microbe was done by 16s rDNA sequencing. The genomic DNA was isolated from the pure culture of the microorganism. The 1.3kb-1.5kb 16s-rDNA fragment was amplified using high–fidelity PCR polymerase. The PCR product was sequenced bi-directionally. Finally, the sequence data was aligned and analyzed to identify the bacterium to its closest neighbours.

C. *Inoculum preparation-*

The pure culture of bacterium was inoculated in 50mL BHI broth. Incubation was done for 120 rpm at 30°C for 48 hours. 1ml of the broth was further transferred into 50ml BHI broth. The culture was then standardized by measuring the optical density of the broth at 600nm. Bacterial culture with an optical density of 0.6-0.8 was used as an inoculum.

D. *Pigment extraction-*

Pigment extraction was done by following the method of Mitra et al. 2016 [10]. Briefly, the bacterial broth was collected and centrifuged at 4°C at 4000rpm for 20 minutes. The resultant cell pellet was washed by suspending it with water. This cell pellet was collected and treated with methanol and centrifuged. Finally the supernatant was collected.

E. *Biomass estimation-*

Biomass estimation was done by using the method of Mitra et al. 2017 [11]. The obtained biomass was expressed in g/L.

III. RESULTS AND DISCUSSIONS

A. *Bacterial identification*

The genomic DNA of the experimental microorganism was isolated for identifying the microorganism based on the 16s rDNA sequence. The sequence consisted of 1266 base pairs. It was observed that the aligned sequence data had 99% match with *Kocuria sp.* J01 (Accession no. KM216829.1). The obtained phylogenetic tree has been shown in Figure 1.

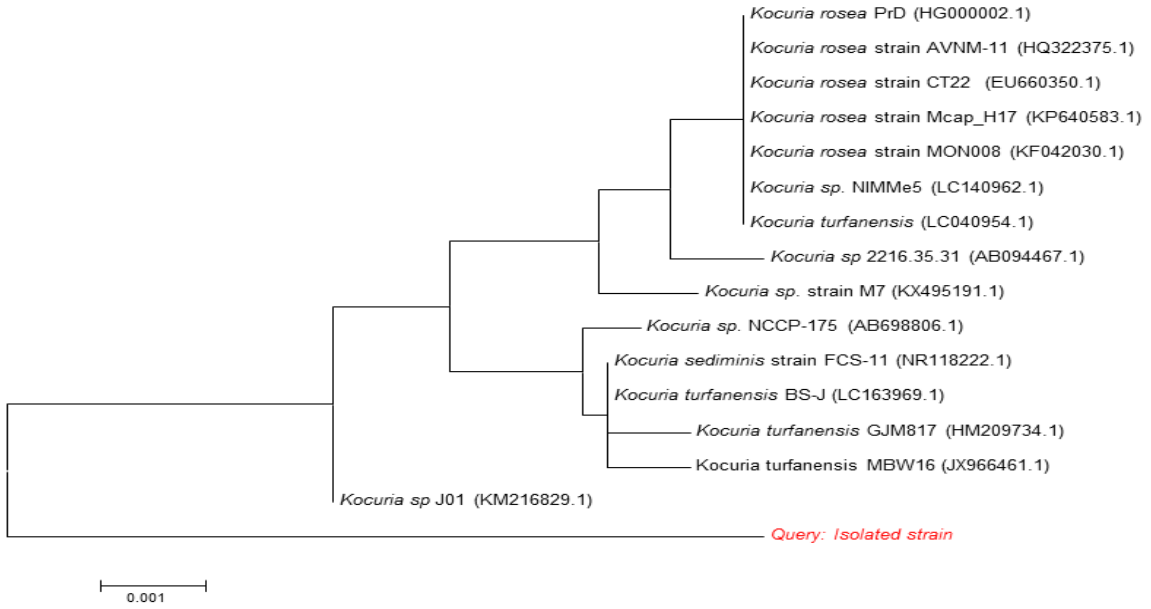

Fig 1 : Phylogenetic tree

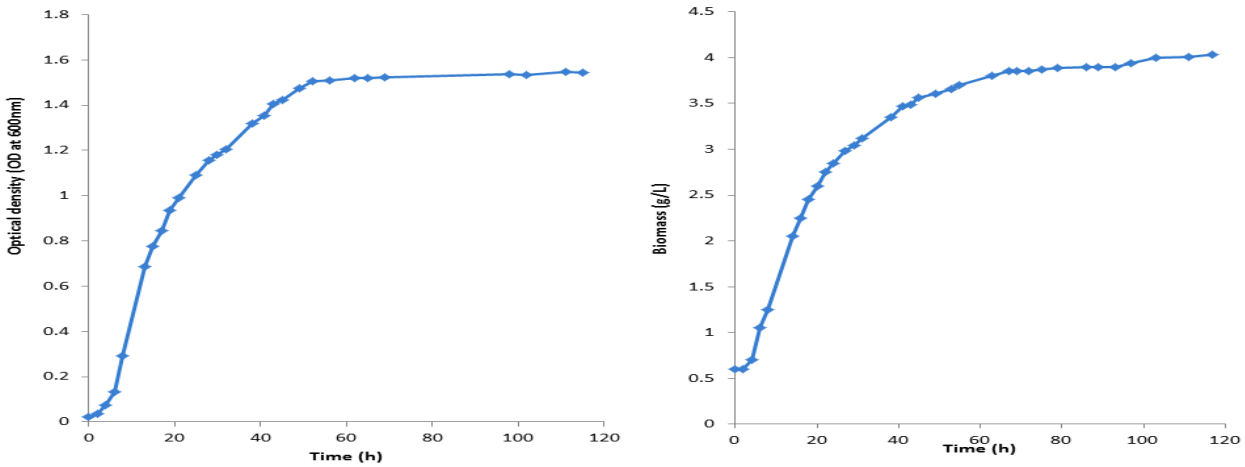

Fig.2 : Growth profile of the isolated bacterium

B. Growth profiling

The growth profile of the experimental bacterium was studied in terms of optical density (λ_{600}) and biomass. Figure 2 shows the typical growth pattern of the bacterium. It was observed that the bacterium had a lag phase of 4h. After 4h, the bacterium entered into the exponential phase which continued up to 50h. After 50h, the stationary phase was achieved.

Spectrophotometric analysis of the pigment

The extracted pigment was spectrophotometrically scanned in the wavelength range of 300-700nm. Different solvent systems were used to study the shift in the absorption spectrum and the distinct peaks were screened. Figure 3 showed the absorption spectrum of the extracted pigment in different solvent system. Similar λ_{max} was observed in methanol, hexane and petroleum benzene. But a shift of 10-20nm was observed in chloroform. This shift was might be due to the change in refractive index of the solvent [12]. From the absorption spectrum, it was predicted that the obtained pigment could be Lycopene(more specifically Carotene).

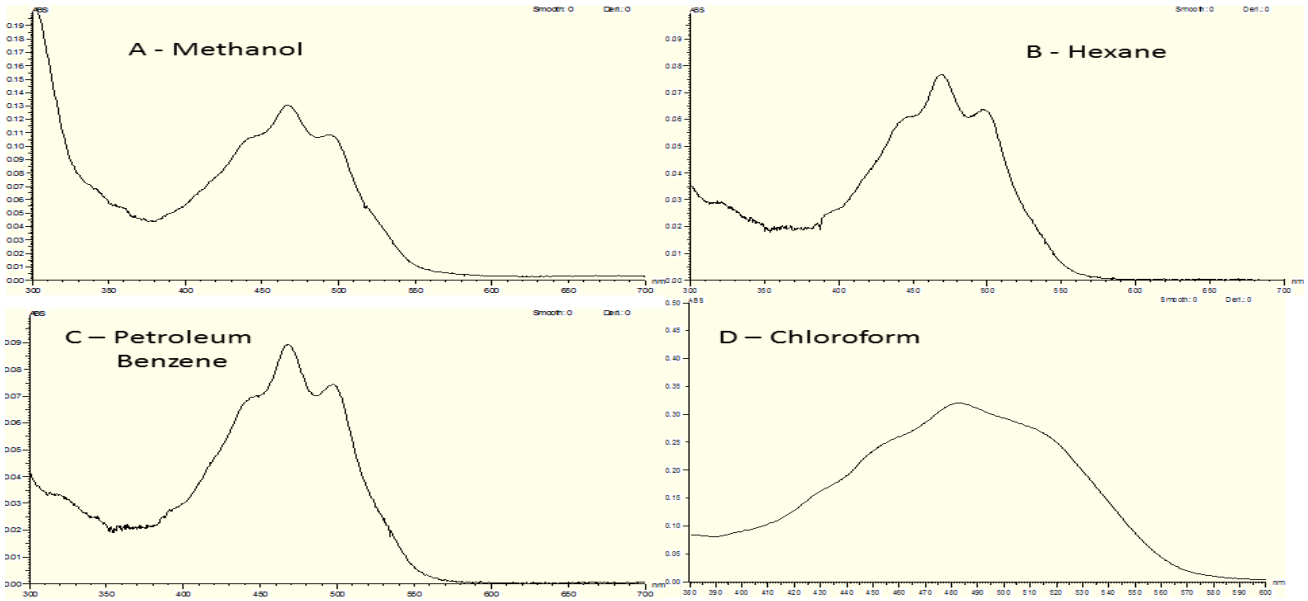

Fig 3: Wavelength scanning of the extracted pigment in different solvent

C. Comparison with tomato extract

Lycopene extract from tomato (*Lycopersicon esculentum L.*) was obtained in petroleum benzene and spectrophotometrically scanned in the wavelength range of 300-700nm. It was observed that the absorption spectrum of the obtained pigment was similar to the spectrum of the lycopene extract of the tomato. Figure 4 shows that the extracted pigment had distinct peaks at 448, 469, 497nm with similar peaks at 444, 468,498 nm of the pigment isolated from tomato. Thus from this study, it was assumed that the extracted pigment could be Lycopene.

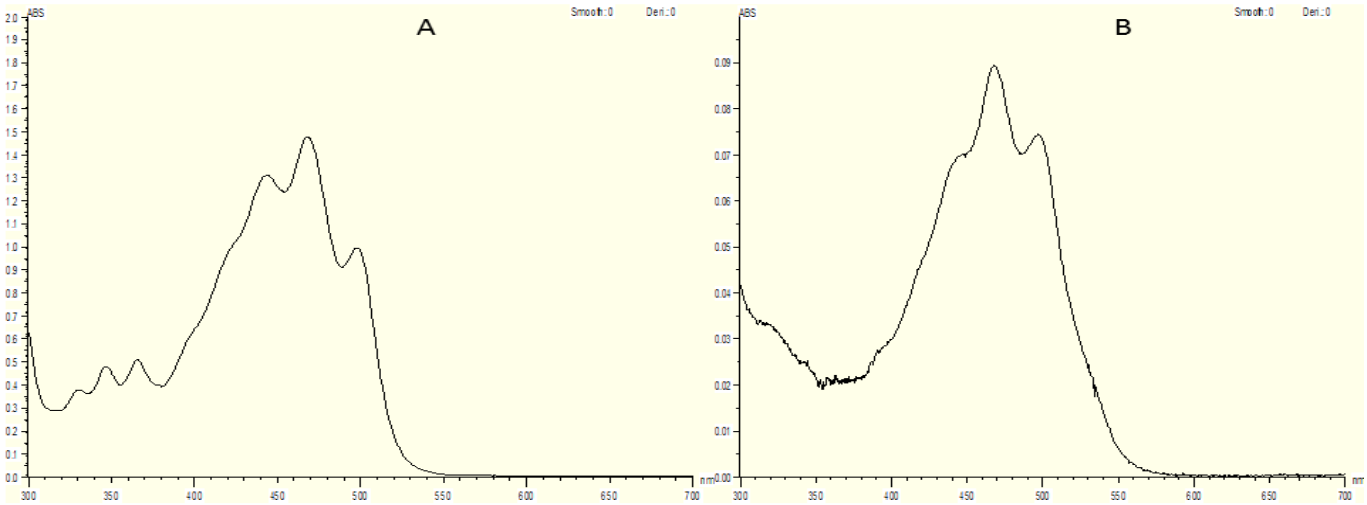

Fig 4: Absorption spectrum of Lycopene extract from tomato (A) and bacterial pigment (B)

D. Time-wise pigment production

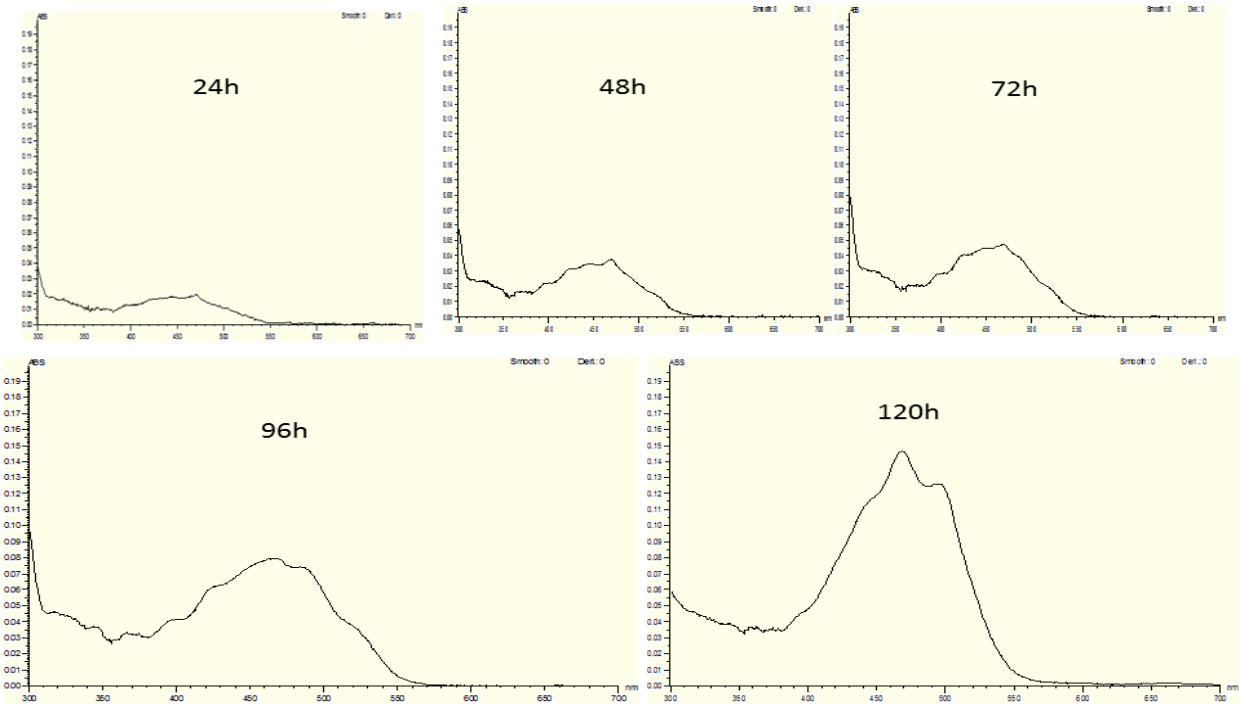

Fig 5: Wavelength scanning of the extracted pigment in different solvent

Spectrophotometric scan of the obtained pigment was studied at different time intervals. It was observed that at 24h, no peaks were formed. But the development of peaks took place from 48h. This showed that the pigment formation took place from 48h onwards. It also indicated that the pigment formation was associated with stationary phase of the bacterial growth. With time, the peaks became sharper. At 120h, distinct peaks were obtained. Figure 5 showed the time-wise absorption spectrum of the extracted pigment.

IV. CONCLUSIONS

In the present study, a pigment producing Gram positive coccus was isolated from soil sample. On 16s rDNA sequencing, it was observed that the isolated bacterium has 99% sequence similarity with Kocuria sp. J01. Preliminary studies revealed that the pigment produced by the newly isolated strain could be Lycopene (i.e., carotenoid). Microbial sources are an attractive alternative to synthetic pigment sources. So, the present study can be a promising contribution in the field of food science and industry.

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