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Research Article

Pigment Production From Bacteria Isolated From Whey

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ABSTRACT

Environmental pollution and population growth necessitate more efficient production processes. Organic whey, which is a low-cost substrate for the food industry, constitutes a promising raw material with its low cost and chemical content for biotechnological processes. This study investigated the pigment production capabilities of bacteria isolated from whey, an industrial waste. Among the isolated bacteria, 4 were determined to be effective pigment producing bacteria. The pigment was extracted from 4 isolates. Pigment characterization was performed by UV spectrophotometer (OD470-OD580) and Fourier Transform Infrared Spectroscopy (FTIR). As a result of the spectrum scanning, it was determined that all pigments gave the maximum absorbance value in the range of 500 nm to 505 nm. In FTIR analysis, all extracted pigments showed characteristic absorption bands of carotenoids between 400 nm and 520 nm. The FTIR peaks obtained from 1469 cm^{-1} and 1726 cm^{-1} regions are known as the fingerprint regions of microbial pigments for biorecolorants. When the results obtained in our study are compared with the literature data, the absorbance values obtained show that the pigment produced is carotenoid and its derivative.

1. Introduction

Pigments are defined as intensely colored chemical covering compounds that are insoluble in water, can absorb light in the visible region, and are used to color various materials. In recent years, producers have focused on the production of natural pigments produced from “plants” and “microorganisms” instead of synthetic pigments, which are potentially harmful to the environment and especially to human health [1]. Since plant-derived pigments have limited water solubility and are not stable against heat and light, microbial pigments attract more attention in the fields of food, textiles, and cosmetics [2, 3].

Bacteria are a good source of pigments. They produce mostly carotenoids, especially β-carotene. *Streptomyces chrestomycetius rubescens* produces lycopene, while *Flavobacterium* sp. has gained importance for its production of zeaxanthin and lutein [2-4]. A mutant strain of *Flavobacterium* can produce zeaxanthin at low temperatures in a base medium with the addition of glucose, corn syrup, and palmitic acid. *Corynebacterium* sp. and *Rhodococcus maris* are also microorganisms adapted for canthaxanthin production [4]. Microbial pigments are preferred due to their features such as reliability in use, medical benefits, being a source of nutrients such as vitamins, production being independent of seasonal and geographical conditions, controllability, wide color range, and efficiency [5].

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In industrial scale, cheap carbon, trace elements, and nitrogen sources are used as substrates. It is important to select sustainable substrates in large-scale microbial production. The use of industrial waste in production is important for both cost reduction and in reducing damage to environmental protection [2-6]. In studies on alternative sources, many different food industrial wastes have been used in pigment production. In this context, paddy straw [7], waste beer [8], beer wort waste [9], orange peels [10], sugarcane [11, 12], rice water [13], sweet potato [14] were used in pigment production.

Whey, which is released at high rates during cheese production, is an important waste for the dairy industry. It is defined as the greenish-yellow liquid by-product that separates from the curd after cutting the curd and remains outside the curd. Nowadays, various whey products such as whey protein concentrates, whey protein isolates, low lactose whey, demineralized whey, and hydrolyzed whey are obtained thanks to technologies such as ultrafiltration, microfiltration, etc. [15].

Whey is a very valuable by-product when processed and turned into powder due to its significant protein, fat, and lactose content, but when released directly into nature, organic substances that can be biodegraded by microorganisms easily cause environmental pollution [16]. Dissolved oxygen in water is used to break down these substances. Since the nitrogen contained in whey dissolves in water, it can mix with groundwater and threaten human and animal health [15, 16]. Approximately 180-190 million tons of whey produced annually poses a major threat to the environment due to its high organic load. In this study, it was aimed to produce and characterize pigments from bacteria isolated from whey.

2. Materials and Methods

2.1. Isolation of bacteria from whey

The whey used in the study was obtained from the cheese factory in Çanakkale province. Bacterial isolation from whey samples transported to the laboratory under appropriate conditions was performed using Tryptic Soy Agar (TSA) (Biolife, 4021502) and Tryptic Soy Broth (TSB) (Biolife, 4021552) media. Isolation and plantings were made in a sterile inoculation (Heal Force, HFsafe 1200LC). After appropriate dilutions, whey samples were taken from 0.1 mL sample tubes and transferred to petri dishes containing the medium, and they were cultivated according to the spread plate method. Petri dishes were incubated at 37°C for 24 hours and bacterial growth was monitored throughout the incubation period. Bacterial samples that showed growth in petri dishes were taken with a sterile loop, and they were inoculated again in petri dishes containing TSA and isolated. Isolated cultures were planted on horizontal agar and short-term stocks were stored at +4°C and long-term stocks were stored at -20°C.

2.2. Determination of active pigment producing isolate

Microorganisms with morphologically colorful colonies among the isolated and purified bacterial isolates were planted in Nutrient Agar (NA) (Lab M, 129472) and incubated for 7 days to be evaluated in terms of pigment production. Pigmented microorganisms were detected among the developing microorganisms. At the end of the incubation period, pigmented colony-forming organisms were transferred to Nutrient Broth (NB) (Lab M, 129491) and incubated at 37°C for 24 hours. Color material production studies were continued with Mineral Salt Medium (MSM) Broth [17].

2.3. Extraction of color matter

Isolates in NB were adjusted to 0.5 McFarland level with 0.9% physiological saline and inoculated into 100 ml MSM Broth. The samples were kept in a shaking incubator at 35°C for 10 days, and at the end of the incubation period, they were transferred to 50 ml sterile centrifuge tubes and centrifuged at 6000 rpm for 15 minutes. The supernatant was discarded and 20 ml of ethyl alcohol solvent was added to the colored cell section at the bottom and kept for 1 night. At the end of one night, the solvent supernatant was cleared of cells by centrifugation at 6000 rpm for 10 minutes and transferred to sterile falcons for spectrum scanning (OD470-580) (Thermo Scientific Genesys 10S).

To obtain dry biomass, colorants were poured into petri dishes in thick layers and left to dry in an oven at 60°C. At the end of 5 days, the dried materials were scraped from the petri dishes and placed in eppendorfs. This obtained dry material was used in all characterization steps [18].

2.4. Microbial pigment spectrum scans

The dried colorants were redissolved in ethyl alcohol and solutions (50, 100, 250, 500, and 1000 μg/ml) were prepared. For the isolates, the spectrum value corresponding to μg/ml of the pigment substance was obtained by measuring the absorbance of the colors given by the pigments at different wavelengths [18].

2.5. Fourier transform infrared spectroscopy (FTIR) analysis

In this study, FTIR analyses were performed to determine the possible groups in the chemical structures of the substances used as pigments. KBr discs prepared with dry pigments were used for FTIR analyses. Powdered pigment samples were mixed with 1% potassium bromide (KBr) and pressed into discs [17]. The FTIR spectrum of the disk is expected to be taken in the region of 400 cm^{-1} - 4000 cm^{-1}.

3. Results and Discussion

3.1. Isolation of bacteria

In the study, 10 Gram (+) and catalase negative isolates that formed colored colonies in whey medium were selected and purified. Microorganisms with morphologically colorful colonies among the purified bacterial isolates were planted in NA and incubated for 7 days to be evaluated in terms of pigment production. Pigmented microorganisms were detected and purified among the incubated microorganisms.

3.2. Identification of active pigment producing isolate and extraction of color substance

Isolates selected according to the colony colors on the NA were inoculated into MSM broth and the color differences in the medium were observed. According to these differences, the isolates with the most prominent pigmentation were selected for microbial pigment production and coded as P1, P2, P3, and P4. To extract pigment-producing bacteria, various methods such as centrifugation (Figure 1), filtration, and ethanol were used to disrupt the cell, and the intracellular pigment was extracted and dried in the oven.

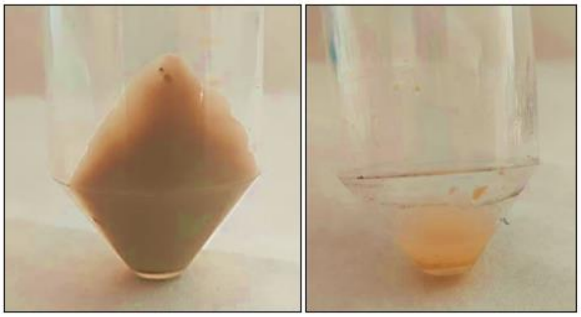

Figure 1. Pigment imageas obtained from isolates

3.3. Microbial pigment spectrum scans

It was determined that the pigments produced from P1 and P4 isolates were light orange, and the pigments produced from P2 and P3 isolates were yellow. For spectrum scanning, dry microbial pigments prepared from isolates coded P1, P2, P3, and P4 were dissolved in the appropriate solvent, and solutions were prepared at concentrations of 50, 100, 250, 500, and 1000 ppm. Readings were made in the appropriate spectrum on the spectrophotometer (OD470-OD580) with the prepared solutions. The most efficient result was obtained at an amount of 500 ppm. The resulting graphs are shown in Figure 2 to 5.

The dry pigments were dissolved in ethanol and spectra were scanned. In the analysis of the samples, especially in the measurements in the UV region, high absorption regions were detected due to compound proteins. As a result of the spectrum scanning, it was determined that all pigments gave their maximum absorbance values between 500 nm and 505 nm. In some studies, it has been determined that quinone molecules have high absorbance points, especially in regions starting from 280 nm wavelengths to 550 nm wavelengths [18]. Different studies have been carried out in the literature for the characterization of pigments. Indra et al., [19] investigated bacterial species that produce high carotenoid pigments on 41 soil samples.

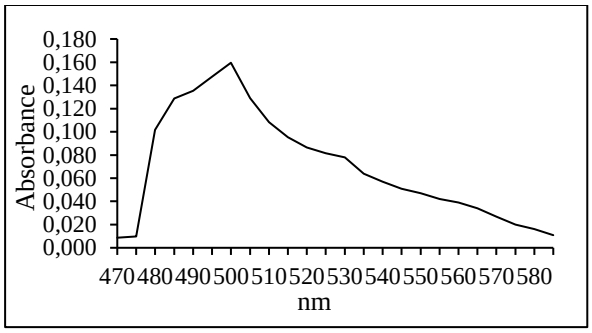

Figure 2. Spectrum scan of P1 coded isolate pigment

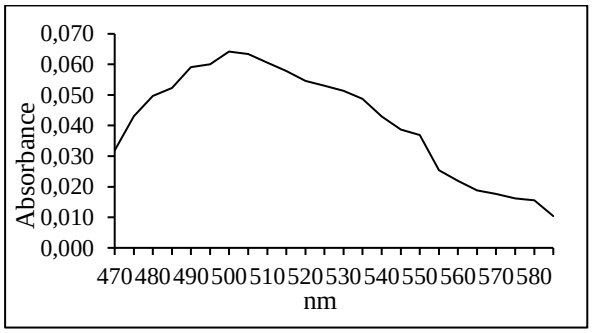

Figure 3. Spectrum scan of P2 coded isolate pigment

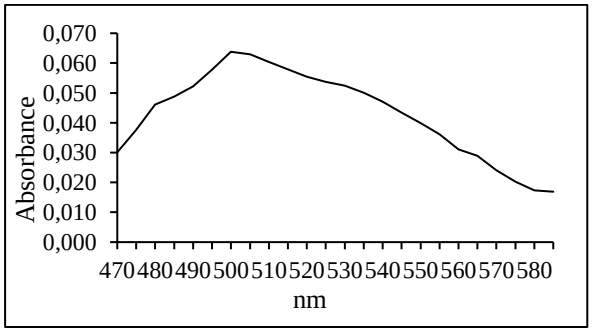

Figure 4. Spectrum scan of P3 coded isolate pigment

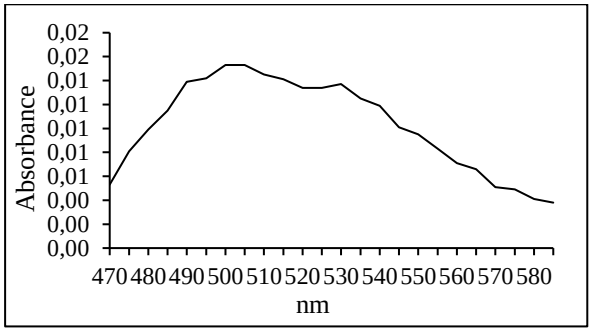

Figure 5. Spectrum scan of P4 coded isolate pigment

As a result of research and bacterial isolation, they obtained 24 bacterial isolates that appeared to produce yellow pigment. These pigments were prepared for spectrophotometric measurements using methanol extract. Evaluations were made as a result of spectrophotometric analysis and the peaks occurring at 450 nm showed the presence of carotenoid pigment. Trivedi et al., [20] isolated bacteria from soil samples. They used methanol, ethanol, and ethyl acetate solvents to isolate pigments from bacteria and performed spectrophotometric analysis and FTIR to characterize the pigments they isolated. As a result of FTIR analysis, it was determined that the pigment obtained was similar to the beta carotene used as standard and that the pigment was a carotenoid derivative. Different spectrophotometric studies have been conducted in the literature. When the results obtained in our study are compared with the literature data, the absorbance values obtained show that the pigment produced is a carotenoid and its derivative [2, 9, 17].

3.4. FTIR analysis

FTIR absorption spectra were determined to characterize the pigment extracts produced by isolates purified from whey. The obtained data are shown in Figure 6 to 9.

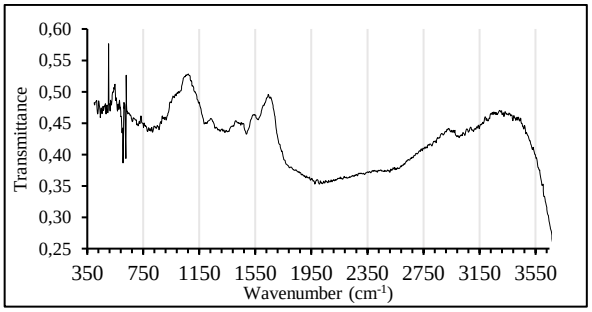

Figure 6. FTIR spectroscopy results of isolate pigment number P1

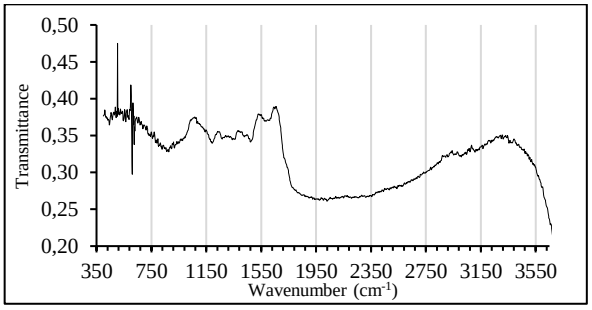

Figure 7. FTIR spectroscopy results of isolate pigment number P2

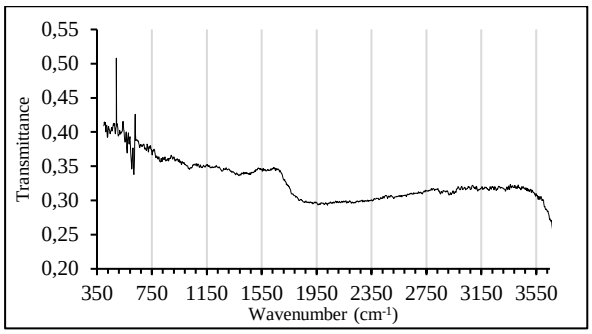

Figure 8. FTIR spectroscopy results of isolate pigment number P3

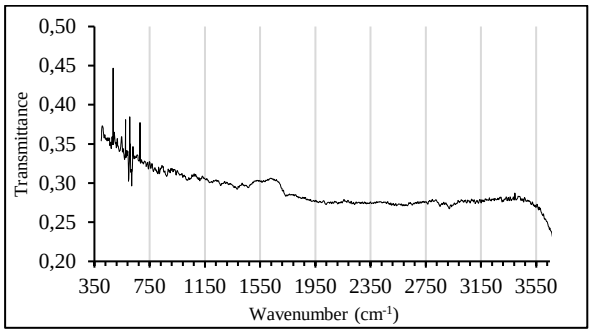

Figure 9. FTIR spectroscopy results of isolate pigment number P4

All extracted pigments showed 400 to 520 nm absorption bands characteristic of carotenoids. The measured spectra correspond to the absorbance of chromophore groups present in the chemical structures of carotenes. β-carotene and zeaxanthin have maximum absorption between 482 nm and 511 nm, depending on the chemical property of the solvent. In addition, the maximum absorbance values determined for lutein are between 440 nm and 503.8 nm. It is thought that the peaks in the range of 1500 cm^{-1} – 1620 cm^{-1} seen in the FTIR graph of pigments P1 and P2 indicate stretching vibrations between aromatic C = C or C = O groups.

The bands in the range of 1320 cm^{-1} – 1390 cm^{-1} of all pigments indicate that the pigment contains pyrrole or indole in its structure. In FTIR spectroscopy of microbial pigments, moderate peaks at 1469 cm^{-1} and 1726 cm^{-1} are known as fingerprint regions for biocolorants. The peaks at 1460 cm^{-1} and 1450 cm^{-1} regions indicate the presence of aliphatic groups, which are organic compounds containing a skeleton in the form of straight or branched chains, formed by covalent bonding of various atoms to each other, in the molecular structures of the pigment material. It is known that the small and continuous peaks seen in the range of 3350 cm^{-1} –3440 cm^{-1} are formed as a result of OH and NH_2 stretching.

In this study, the pigment producing ability of bacteria isolated from whey was investigated and the basic characterization of the produced pigment was carried out [4-6, 15].

Ahmad et al., [21] pigment-producing bacteria *Serratia marcescens*, *Streptomyces coelicolor* and *Thialkalivibrio versutus* were isolated and their prodigiosin production abilities were investigated. The characterization of the obtained pigment was evaluated in terms of physical and chemical properties by various methods such as FTIR and UV-vis Spectroscopy.

Órdenes-Aenishanslins et al., [22] pigment production abilities of psychrotolerant bacteria isolated from soil were investigated using UV-Vis spectrophotometry and FTIR methods. Red and yellow pigments were produced from bacteria identified as *Hymenobacter* sp. and *Chryseobacterium* sp.

Atalay, [23] produced red pigment with bacteria isolated from waste beer and carried out optimization studies. A comparison of the results obtained in our study with literature data shows that the pigment produced is a carotenoid and its derivative. It is envisaged that, with more detailed studies, a raw material with commercial potential and use will be revealed.

4. Conclusion

Although natural pigments were previously produced from plants, microbial pigments have begun to replace vegetable pigments rapidly, as they are not affected by weather conditions and grow quickly and easily. The interest in microbial pigments, which were not preferred at first due to their effects on human health and being more expensive than synthetic ones, increased as it was revealed that they did not have any toxic properties and their prices became competitive with synthetics thanks to the use of cheap raw materials in developing biological processes. Microbial pigments not only grow rapidly and are not affected by environmental conditions, but also the fact that industrial and chemical wastes can be used to grow the

microorganisms used is an extra advantage due to their contribution to environmental pollution. Microbial biotechnology, which is developing day by day around the world, will continue to play a role in reducing environmental pollution by transforming waste into usable products due to the increasing need for cheap raw materials in the future. An increase in microbial diversity due to the research of new microorganisms that can be used in these areas, and biological processes developed for the naturalization of many products will lead to progress in the field of biotechnology and the production of healthier products.

This study examines the presence of Gram (+) microorganisms capable of producing pigments in whey, a by-product of cheese production. With increasing demand across all industries, the use of pigments derived from natural sources is gaining importance. Therefore, the discovery of potential pigment-producing microorganisms in by-products such as whey, which are considered waste, could be a significant step towards meeting the demand for natural colorants across various industries.

Microorganisms are valuable resources used in the production of various industrial products in biotechnological applications. In this context, the discovery of pigment-producing microorganisms in waste products such as whey could contribute to sustainable production processes. Using pigments derived from natural sources offers a more environmentally friendly and sustainable option than synthetic alternatives.

Furthermore, the biodiversity and adaptation capabilities of microorganisms provide a wide potential for synthesizing different pigments in industrial applications. Therefore, the identification and characterization of pigment-producing microorganisms in waste products like whey represent a crucial step in the discovery and development of new and diverse natural colorant sources.

In conclusion, this study not only identifies the presence of Gram (+) microorganisms capable of producing pigments in whey but also emphasizes their significance as valuable resources to meet the demand for natural colorants across all industries. These findings could contribute to further understanding the use of natural pigment-producing microorganisms in future research and the development of more sustainable colorant options in industrial applications.

Article Information Form

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Authors' Contribution
The authors contributed equally to the study.

The Declaration of Conflict of Interest/ Common Interest
No conflict of interest or common interest has been declared by the authors.

The Declaration of Ethics Committee Approval
This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics
The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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