Biotechnology Reports 4 (2014) 139–146

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

Production of melanin by soil microbial isolate on fruit waste extract: two step optimization of key parameters

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ARTICLE INFO

Article history:
Received 2 April 2014
Received in revised form 10 September 2014
Accepted 3 October 2014
Available online 7 October 2014

Keywords:
B. safensis
Photoprotective
Radical scavenger
Metal chelator,

ABSTRACT

In this study, optimization of production parameters influencing melanin production in an economical fruit waste extract was attempted using a garden soil isolate (*Bacillus safensis*). Taguchi approach was adopted for screening of critical parameters and further optimization was done using a central composite design of response surface methodology (RSM). At optimum conditions (pH 6.84 and Temp 30.7 °C), a significant yield of ~6.96 mg/mL was observed. Statistical analysis revealed that the experimental results fitted well to the statistical model with model R^2 value 0.982. The optimization of process parameters using RSM reported a 15% increase in the pigment yield than average yield obtained from the studied model. The melanin produced was confirmed by UV–visible spectroscopy, FTIR and XRD analysis. Moreover melanin obtained has significant photoprotective, radical scavenging and metal chelating activity. Thus, *B. safensis* has the potential to be a new source for the production of melanin, which is of industrial interest.

1. Introduction

Melanins are the natural pigments which have their presence in animals, plants and in most of the microorganisms [1]. They are the dark coloured negatively charged high molecular weight pigments which are formed due to polymerized phenolic and/or indolic compounds. These complex polymers are amorphous in nature and shows solubility in neither aqueous nor organic solvents. They showed resistance to concentrated acids and are susceptible to bleaching by oxidizing agents [2]. They play a vital role in defence and protection mechanisms that improve the survival and competitiveness of the organisms [3]. Melanin is known for its absorption capacity of radiation of all wavelengths with an optimum absorbance at UV range [4] which prevents photo induced damage. Hence it is used in the preparation of photo absorbing optical lenses and in bioplastics. Besides photo protection it has versatile biological activities such as radical scavenging, antioxidant, antitumor, anti-inflammatory [5] and as immune stimulating agent [6].

Melanin obtained from microbes has great advantages over melanin from animals and plants. Microorganisms don't cause the problems of seasonal variations and are selected arsenals as they modify them according to the medium and conditions provided to them [7]. Targeting melanogenisis in microbes may help to discover antimicrobial drugs. For example, melanins produced by *Cryptococcus neoformans* and *Burkholderia cepacia* offer virulence and contribute to the growing resistance of these pathogenic bacteria towards antibiotics [2,8]. The melanin synthesized by microbes shows metal chelating ability too (sorb the radioactive wastes uranium) [9]. There are reports that showed the anti HIV properties of melanin and their role in photo voltage generation and fluorescence studies [10–11]. Therefore, all these properties of melanin make them unique and are widely used in cosmetic, sunscreen protection creams, eye glasses, pharmaceuticals, and food industries

Considering the potential uses and increasing demand for the melanin pigment there is a need to conduct studies on the production of melanin from microbes. There are reports on melanin production from various microorganisms, including *Bacillus* species which are well known for their pigment production ability in various stress environments [4,12]. Selection of substrate for melanin production has economic importance. For instance till date expensive substrates like NCM media [4], LB (Luria–Bertani) media [12], minimal media supplemented with L-tyrosine [13], amino acids enriched tryptone broth agar [14] and so on [15–16] were used for high yield of melanin. Owing to the economy and practicability of the melanin production process; the need to use economically feasible substrates along with optimization of the key parameters is needed. In recent years, considerable interest

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http://dx.doi.org/10.1016/j.btre.2014.10.001

has been developed in using agro-industrial wastes as substrates for valuable products like pigments. The abundantly available fruit waste in India used widely as animal feed or disposed to the soil. The effective utilization of this waste which is rich in carbohydrates and other nutrients can address our primary objective of melanin production in a cheaper way. An optimization strategy like Taguchi method [17] is a systematic technique of design and analysis of experiments that has been employed successfully in recent years to design, improve the product quality economically [18], and a central composite design (CCD) approach has been used to fit a polynomial model. The complementary use of both the methodologies provides a great amount of information based on only a small number of experiments and to scheme a process.

In this study, a bacterium capable of producing melanin was isolated from garden soil and subsequently characterized. The strain was cultivated on the fruit waste extract (FWE) as the sole source of energy to produce significant amounts of melanin. The key parameters in melanin production were identified and optimized using simple two steps Taguchi and CCD (central composite design) approach. Upon purification and characterization, the obtained melanin was tested for *In vitro* sun protection effect, free radical scavenging and metal chelating activities.

2. Materials and methods

2.1. Chemicals and microorganism

DPPH (2,2-diphenyl-1-picrylhydrazyl), purchased from HiMedia chemicals, Mumbai, India. Ascorbic acid was purchased from Merck, India. Ferrozine and melanin (synthetic) were purchased from Sigma–Aldrich, India. Ethanol, NaCl, NaOH, HCl are from Merck, India and all other chemicals used were of analytical reagent grade throughout the study. Ultrapure water was used for the experiments and aseptic conditions were maintained wherever necessary.

The microorganism used in this study was isolated from garden soil in front of Department of Chemical Engineering, National Institute of Technology, Rourkela, India using serial dilution technique on a nutrient agar (NA) [19]. Using 10^7 dilution of soil sample on NA plates, the melanin producing organism was identified. It was separated by observing a diffusible black pigment on NA plates after 24 h. The isolated culture was preserved on NA slants at 4 °C and sub-cultured at monthly intervals.

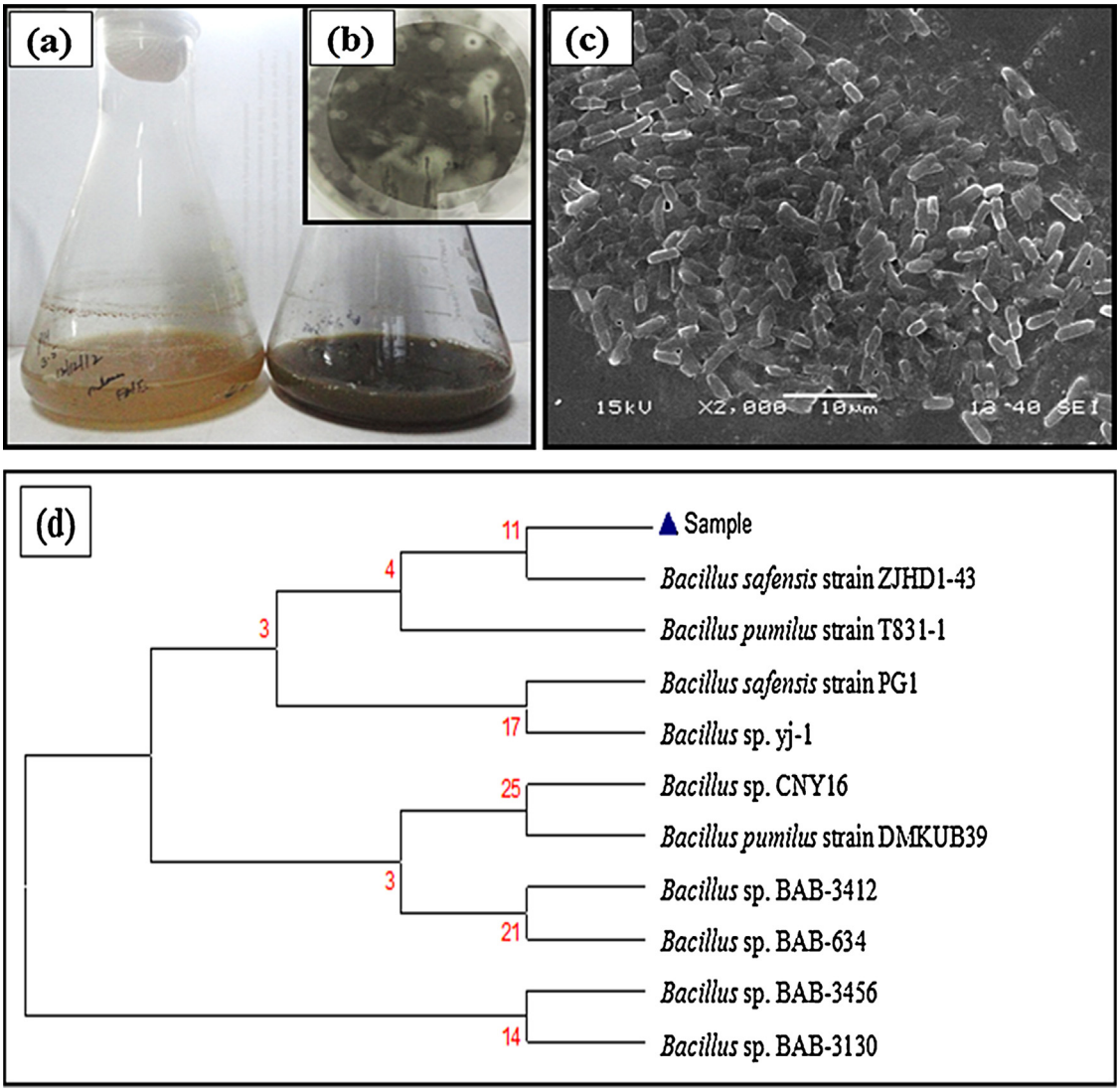

Fig. 1. (a) FWE before (left) and after melanin production (right) by the garden soil microbial isolate, (b) colonies with diffusible melanin on NA plates, and (c) SEM image of the microorganism. (d) Phylogenetic tree showing the position of the isolate ZJHD1–43 with reference to related strains.