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RESEARCH ARTICLE OPEN ACCESS

Isolation and Elucidation of Bacterial Melanin's Sun Protection Factor (SPF) for Photoprotection in Cosmetics

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Abstract

The outline of our work delineates the isolation and evaluation of sun screening activity of melanin producers such as *Pseudomonas mosselli* STGRDS1, *Pseudomonas putida* STGRDS3, *Bacillus amyloliquefaciens* STGRDV11, *Bacillus subtilis* STGRDV5 and *Bacillus cereus* STGRDT12. All of the isolates were tested against the fungal melanin STGRDM1, which was used as control throughout the study. The Sun Protection Factor (SPF) of formulated creams containing 5% and 10% of melanin was determined with values ranging from 1.96 ± 0.008 to 26.33 ± 0.061; further, the transmission spectroscopy was used to calculate the percentage of protection factor that stipulates the potentiality of pigments showing sunscreen effect.

Keywords: Melanin, *Pseudomonas mosselli, Pseudomonas putida*, *Bacillus amyloliquefaciens*, *Bacillus subtilis, Bacillus cereus,* SPF, Transmission Spectroscopy

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INTRODUCTION

Melanin is classified as polyphenol compounds, which exhibits brown, grey and black pigments in plants, microbes and animals. Foremost groups of melanin includes, Eumelanin that discerns as brown to black in color and this type of melanin is contemplated to be the conventional type that is extensively found in vertebrates and invertebrates. Pheomelanins are found in birds and mammals that are characterized as yellow or red in color. Allomelanins are chiefly found in seeds, fungi and spores. Melanin production has been contemplated in both Prokaryota and Eukaryota.[1] Harmful effects are caused by the UV region. Skin that is detriment to elasticity and the collagen fibers of connective tissue is caused by exposure to UV A radiation that results in premature ageing (photo ageing).[2] In contrast, acute inflammation (sunburn) and extreme sunburn are caused by exposure to UV B radiation. Before reaching the earth surface, radiation from UV C is conventionally filtered by the atmosphere; radiation from UV B is only partially filtered by the ozone layer. Skin cancers are mainly caused due to UV radiations.[3]

Melanin plays an essential role in photoprotection, acting as a physical barrier absorbent filter that prevents UV penetration into the epidermis. Early in the 20th century, sunscreen usage became widespread. Initially, sunscreen preparation was done with salicylates, which were reported for allergic, photoallergic reactions, contact dermatitis, severe anaphylactic reactions, photo-toxic, and contact urticaria. Therefore, there is a high demand for natural sunscreen that would be effective with less or no side effects. A regimen that comprehends effective sunscreen and clothing helps in photoprotection. Two major pathways that involves sunscreen activity are (i) absorption (ii) UV energy scattering and reflection.[4] Sunscreen efficacy is calculated by Sun Protection Factor (SPF). UV energy gets explicated by SPF that is required in protecting the skin to produce the Minimal erythema dose (MED) divided by the UV energy imparted in unprotected skin to produce the same MED.[5] It is assumed that the sunscreen activity of melanin ranges from 1.5 to 2.0 SPF to 4 SPF, indicating that melanin can absorb 50 to 75% of UV radiation.[6] Enhancement of SPF study in melanin determines the efficacy in cosmetic applications. The purpose of the present study was to identify the best melanin producers, evaluate their photoprotective activity via in vitro SPF determination, and calculate their average UV-A and UV-B protection factor via transmission spectroscopy.

MATERIALS AND METHODS

Chemicals and bacterial isolates

In this work, the chemicals L-tyrosine and fungal melanin (Mykotech, Goa) were used. Both are analytical reagent grade chemicals (Hi-media Laboratories Pvt Ltd, Mumbai, Maharastra, India). Soil was the main place where melanin producers were found. They were sorted out using the serial dilution method, and the colonies that made pigment were then grown in tyrosine broth.[21] Biochemical analysis enabled the identification of the bacterial isolates, which was followed by 16S rDNA sequencing and analysis. The strains were sequenced and preserved at the National Center for Biotechnology Information (NCBI).[7-9]

Melanin extraction

Tyrosine basal broth was used as a production medium in the study.[10] Primary inoculum, such as melanin-producing culture, was added to 50ml of production medium, which was kept in environmental shaker at 140rpm at 37˚± 2˚C for 180h. After which, the supernatant was acidified to pH 2 using 1N HCL. The purified melanin was extracted by adding acid, water, and ethanol, and then drying the mixture. [11]

Determination of sun protection factor

Purified melanin was formulated with 5% and 10% cream by adding 0.5ml and 1ml of bacterial melanin as stock solution. To 10ml of ethanol, 10mg of stock was added and serially diluted to 1000 µg/ml, 500 µg/ml, 250 µg/ml, and 125 µg/ml concentrations as working stock. The absorption was determined from ranges 290nm - 320nm and ethanol was taken as blank. The data evaluated was equated in accordance with the Mansur equation.[12]

$$SPF\ (spectrophotometric) = CF \times \sum_{320}^{290} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

SPF determination by transmission spectrum

A polyvinyl chloride (PVC) sheet strip was taken, and the formulated cream was spread on it as a thin film with concentrations of 5% and 10%. This was put inside a UV-Vis cuvette with the clear side facing out, and the transmission spectrum was measured from 290nm to 400nm using air as a standard. Further, UV A and UV B protection factors were elucidated with the following formulas.[13]

1) Determination of UV A or UVB blocking percentage.

$$T\ (UVA)\ avg = \frac{^{400}\Sigma_{320}\ T\ \lambda\ X\ \Delta\lambda}{^{400}\Sigma_{320}\ \Delta\lambda}$$

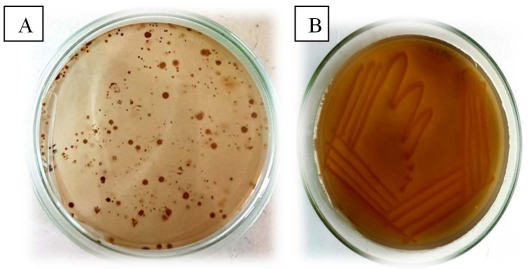

Figure 1. Primary screening for melanin producing bacteria showing formation of colonies of very dark brown colour (a) and Melanin producing isolate streaked in tyrosine basal agar medium showing utilization of tyrosine (b)

$$T\ (UVA)\ avg = \frac{^{320}\Sigma_{290}\ T\ \lambda\ X\ \Delta\lambda}{^{320}\Sigma_{290}\ \Delta\lambda}$$

100-T(UVA) or T(UVB) gives % blocking or % protection against the UVA or UVB.

2) Evaluation of average UVA protection factor (PF)

$$PF\ Am = \frac{^{400}\Sigma_{320\ MPF}\ T\ \lambda\ X\ \Delta\lambda}{^{400}\Sigma_{320}\ \Delta\lambda}$$

Statistical analysis

All experiments were repeated twice and are expressed as mean ± standard deviation. Microsoft Excel was used for statistical analysis.

RESULTS AND DISCUSSION

The isolates were treated with primary

Table 1. Normalized Product Function Used in SPF Calculation

Wavelength (λ nm)	EE(λ) x I(λ) (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

Table 2. Determination of Sun Protection Factor at various concentrations for 5% and 10% formulated cream of melanin

Concen. (µg/ml)		M1	S1	S3	V11	V5	T12
5%	125	1.96 ± 0.008^{aA}	2.75 ± 0.047^{aA}	4.05 ± 0.038^{cA}	4.00 ± 0.051^{bA}	6.39 ± 0.482^{cA}	2.77±0.071^{bA}
	250	3.76 ± 0.019^{bA}	3.65 ± 0.050^{bA}	7.23 ± 0.124^{cA}	7.12 ± 0.050^{bA}	7.89 ± 0.906^{dB}	3.64 ± 0.034^{aA}
	500	5.45 ± 0.225^{bA}	4.69 ± 0.043^{bA}	15.10±0.041^{bA}	15.02±0.016^{bA}	12.07±1.022^{bB}	4.02 ± 0.898^{aB}
	1000	7.70 ± 0.029^{bA}	5.85 ± 0.050^{bA}	21.75±0.050^{cA}	21.68±0.076^{bA}	14.25 ±1.165^{bC}	5.84 ± 0.034^{aC}
10%	125	3.69 ± 0.177^{aA}	7.52 ±0.041^{bA}	4.33 ± 0.041^{bA}	4.26 ± 0.060^{aA}	8.45 ± 0.066^{cA}	7.54 ± 0.059^{cA}
	250	15.76±0.067^{cA}	10.58±0.048^{cA}	8.33 ± 0.054^{bA}	8.27 ± 0.041^{aA}	17.19 ±6.917^{dB}	10.57±0.034^{bA}
	500	17.10±0.048^{dA}	14.97±0.115^{aA}	17.05±0.112^{cA}	16.94±0.050^{bA}	23.79±10.409^{eC}	14.99±0.086^{bA}
	1000	19.10±0.053^{aA}	23.57±0.074^{aA}	26.06±0.067^{cA}	26.00 ± 0.055^{bA}	26.33 ± 0.061^{cD}	23.58 ± 0.095^{bA}

Each value in the table is represented as mean ± SD (n=3). a, b, c, d, e values in rows with different letters are significantly different at p≤ 0.005. Values in the same column within concentrations are followed by different letter (a-c) are significantly different at p≤ 0.005

and secondary screening to check for melanin producers. Whereas with primary screening, the culture is streaked onto tyrosine agar plates (Figure 1) and with secondary screening, melanin producers are inoculated in tyrosine basal broth for its production (Figure 2). STGRDS1 and STGRDS3 were isolated from garden soil, STGRDV11 and STGRDV5 from vermicompost soil, and STGRDT12 was obtained from tomato-yielding soil as part of the preliminary screening for melanin producers in soil. Pigments with a clear zone on tyrosine agar plates were designated as melanin producers, and secondary screening was done to determine the ability of melanin producers to produce in tyrosine basal broth, which serves as the production medium. All the strains showed good production and were therefore selected for further application studies. The cultures were then taken up for molecular identification by 16s rDNA sequencing and the identity of the sequence was searched against the GenBank database using the NCBI BLAST tool and their accession numbers were obtained. STGRDS1 *Pseudomonas mosselli* (MN967075); STGRDS3 *Pseudomonas putida* (MT006089); STGRDV11 *Bacillus amyloliquefaciens* (MW629851); *Bacillus subtilis* (MW674644); and *Bacillus cereus* (MW674663) were identified as melanin producers by 16srDNA sequence analysis. Melanin pigment producing dark colonies in Pseudomonas sp were observed in tyrosine basal agar.[14] Bacillus cereus melanin production was evaluated on nutrient broths, LB agar, nutrient

Table 3. Determination of percent protection of formulated cream against UV rays analysis

Culture	Formulation	% protection against UV A	% protection against UV B	Average UV A protection factor	Average UV B protection factor
M1	5%	84.55 ± 0.141^{A}	91.01 ± 0.162^{D}	15.45	8.99
S1		54.52 ± 0.021^{A}	87.69 ± 0.014^{D}	45.48	12.31
S3		63.67 ± 0.029^{A}	90.17 ± 0.090^{D}	36.33	9.83
V11		62.29 ± 0.212^{A}	97.27 ± 0.101^{A}	37.71	2.73
V5		74.87 ±0.399^{A}	91.10 ± 0.029^{C}	25.13	8.9
T12		23.90 ± 0.062^{A}	92.06 ± 4.721^{B}	76.1	7.94
M1	10%	87.94 ± 0.014^{A}	95.03 ± 0.028^{A}	12.06	4.97
S1		82.98 ± 0.014^{A}	88.92 ± 0.035^{A}	17.02	11.08
S3		51.43 ± 1.158^{A}	86.57 ± 0.085^{A}	48.57	13.43
V11		53.62 ± 0.382^{A}	87.52 ±0.121^{A}	46.38	12.48
V5		92.18 ± 0.282^{A}	72.42 ± 0.101^{A}	7.82	27.58
T12		89.36 ± 0.116^{A}	79.15 ± 0.191^{A}	10.64	20.85

Each value in the table is represented as mean ± SD (n=3). Values in the same column within concentrations are followed by different letter (A, B, C, D) are significantly different at p≤ 0.005

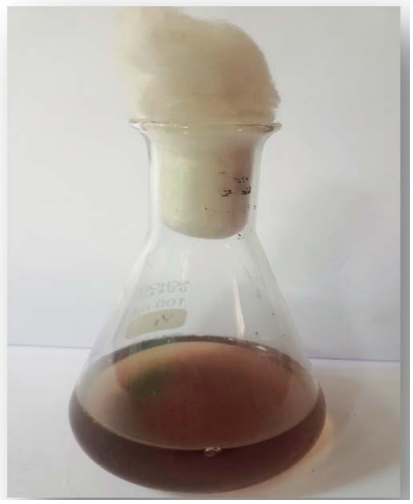

Figure 2. Secondary screening of STSGRDS1

Table 4. SPF classification table

Protection level	SPF value
Low protection	6, 10
Medium protection	15, 20, 25
High protection	30, 40
Very high protection	50+

agar, and T3 agar, which displayed a blackish-brown pigment throughout the medium.[15] Compared to previous reports, it was discovered that by optimizing culture conditions, the production of melanin could be increased from a white medium to a dark brown medium upon completion of melanin production.[16-18] The extracted melanin was purified and weighed as follows; S1 weighed 0.211g, S3 weighed 0.112g, V11 weighed 0.114g, V5 weighed 0.115g, T12 weighed 0.105g. This purified melanin was used for further application study.

The ability of the Sun Protection Factor (SPF) was checked with the cream formulated with different concentrations, such as 5% and 10%, by purifying bacterial melanin in accordance with the Mansur equation method. (Table 1) in the range of 290nm to 400nm. Melanin producers such as S1, S3, V11, V5 and T12 were compared to the standard melanin M1. Determination of SPF of all purified melanin such as S1, S3, V11, V5 and T12 has compared with standard M1, proving that all the melanin producers showed enhanced protection in both 5% and 10% formulations (Table 2). All the bacterial melanin samples used in the study were observed to enhance the SPF values of commercial creams, thereby providing more protection against harmful radiations such as UV radiation. Enhancement of SPF values was reported in *Bacillus safensis, Cinnamomum burmannii* and *Osmanthus fragrans*.[19] It was previously reported that Dietzia schimae obtained SPF of about 20.22, *Pseudomonas koreensis* strain expressed 61.55.[20,21] In another study, Sun protection factor of fungal melanin was compared with pure cream that showed 1.0 and melanin blended SPF showed 2.5, showing its photoprotection ability.[22] SPF values of various concentrations such as 120µg/ml, 40 µg/ml and 60 µg/ml of water fraction, ethanol extract and n- butanol fraction of *Chromolaena odarata* leaves were evaluated, which showed SPF ranges of about 2 to 4 and with lesser concentrations, SPF values were lesser than 2.[23] Various coffea such as Coffea Arabica, Canephora and Liberica were formulated and they were screened for Sun Protection activity which showed very good protection; 36.087 ± 0.0005; 35.007 ± 0.0005; 36,867 ± 0.0005 respectively.[24] Melanin formulated in cream was further checked by transmission spectroscopy *in vitro* activity with UV - Vis spectrometer ranging from 290nm to 400nm to calculate the percentage of protection (Table 3). Formulated cream containing various concentrations of the leaves extract of *Butea monosperma* was determined through the transmission spectroscopy method, proving that with the increase in the concentration of extract, there is an increase in the protection from the UV radiation and average UV - A protection factor.[25-27] The SPF classification table shows the ranges of protection (Table 4). In previous reports, synthetic skin was used to assess the sunscreen efficacy by employing the assessment of transmission spectroscopy, that showed reduced transmission spectrum with increasing concentrations after 2h of application.[28] In a study, the correlation of absorbance and transmittance were used to evaluate the SPF of sunscreen and blockage of UV radiation and the results acclaimed that the SPF of various ranges from (15, 20, 24, 30, 50 and 60) revealed that the SPF and sunscreen absorption had a direct relationship.[29] According to a study, the imaging of a sunscreen was evaluated by transmission spectroscopy within the emulsion that made it possible to move outside the spectral region of the visible light and with UV, image with different SPF ingredients within the formulations were done first time with optical microscopy.[30] In recent study, an active ingredient melanin/ TiO2 nanoparticles were used in formulating sunscreen that achieved SPF of about 116.9 and 162.4 with about 10 percentage of weight and 15 percentage of weight.[31] Also, ethyl acetate of *Padina boergesenii* proved to be a great potential as a natural UV filter in a specific sunscreen formulation.[32] The formulation of cream of *Polycladia myrica* with five percentage of ethyl acetate fraction expressed a high SPF of ranges 31.79 ± 4.73, UVA/PF (24.67 ± 4.03), critical wavelength (383.2 ± 0.1nm) and UVA and UVB ratio (0.98 ± 0.01) revealed that these extracted formulation proved to be valuable sun protective emulsion.[33]

Each value in the table is represented as mean ± SD (n=3).[a,b,c,d,e] Values in rows with different letters are significantly different at p≤ 0.005. Values in the same column within concentrations are followed by different letter ([a-c]) are significantly different at p≤ 0.005.

Each value in the table is represented as

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All data sets generated and analyzed during the study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

CONCLUSION

This study revealed the screening of melanin producers and sun protection factors by purified melanin producers. This study could be of use to provide information before beginning with in vivo studies. In the future, this potential melanin producers to develop higher SPF creams or lotions that could provide efficient photoprotective activity.

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