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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF MELANIN PRODUCED BY A *Pseudomonas balearica* STRAIN

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Abstract- In this paper, a water soluble melanin is produced by *Pseudomonas balearica* strain U7, isolated from the marine green alga *Ulva lactuca.* Up to 110 mg/l of melanin is produced in the extracellular semi synthetic medium, typically containing L-tyrosine as sole carbone source. The quantity and structure of the extracted melanin were determined using UV-visible, FT-IR and ^{1}H NMR. This melanin showed an antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and the phytopathogenic strains *Erwinia chrysanthemi* and *Erwinia carotovora*. In addition, the extracted melanin exhibited considerable scavenging activity of the stable free radical DPPH. This is the first report on characterization of melanin produced by a novel strain of *Pseudomonas balearica* and its antibacterial effect against a phytopathogenic bacteria. Our study suggests that the strain *P. balearica* is a potential source of antioxidant melanin production for cosmetic and pharmaceutical uses while the use of *P. balearica* as potential biological control against phytopathogenic *Erwinia* should be exploited in agriculture.

Keywords- melanin, L-tyrosine, FT-IR, 1H NMR, antioxidant activity, antimicrobial activity, Erwinia, *Pseudomonas balearica*

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Introduction

Melanins are amorphous black pigments of high molecular weight and mostly hydrophobic [1]. They are formed by oxidative polymerization of phenolic or indolic compounds present in animals, plants, bacteria and fungi [2].

Melanin synthesis has been reported in various bacteria, including actinomycetes [3], recombinant *Escherichia coli* W3110 coding for a tyrosinase [4], wild-type *Bacillus cereus* [5], *Bacillus thuringiensis* mutant [6], *Klebsiella sp.GSK* [7], *Pseudomonas stutzeri* [8] and recently by *Azotobacter chroococcum* [9]. Some fungal species are also able to synthesize melanin, including *Aspergillus fumigates* [10], *Aspergillus Bridgeri* [11], *Pneumocystis carinii* [12] and *Cryptococcus neoformans* [13]. Melanins are not considered essential for growth and development of these microorganisms, but are required to enhance their ability to compete and survive in unfavourable environmental conditions, such as under UV radiation [14].

Melanins are a class of pigments which have attracted great interest, especially in cosmetics to protect against the noxious effects of UV radiation by incorporation in sun-protection compounds [15]. In medicine, melanins have a very effective radical scavenging capacity [16], and they have been proposed for protecting patients undergoing radiation treatment against the harmful effects of gamma rays used in cancer therapy [17].

On the other hand, melanins could be also used as semiconductors in bio-electronic sciences development [18]. Currently, melanins produced naturally, mainly from sepia and fungi, are insoluble in water and are extracted under harsh procedures, or by chemical synthesis by converting melanin precursors such as tyrosine and L-DOPA, using chemical oxidizing agents. For this reason, various technologies including genetically engineering have been carried out worldwide to develop easier processes for over producing water soluble melanin [4,19]. However; the developed methods were expensive, resulting in high cost of melanin, and production of compounds from genetically engineered organisms requires restrictions based on regulations.

Furthermore; marine microbes offer great opportunities for biodiscovery, they could provide a new resource for structurally diverse secondary metabolites like melanin. The seaweeds represent an overlooked habitat from which to isolate these important microorganisms [20,21].

To overcome above limitations for the production of melanin, the present study relates to isolation and characterization of a *Pseudomonas balearica* strain from the green alga *Ulva Lactuca*; able to use and convert L-tyrosine to a natural water soluble melanin. The

melanin is freely secreted into an economical culture medium M9 with L-tyrosine. The melanin produced by this bacterium was characterized according to its spectroscopic and chemical properties. Further, the free radical scavenging and antimicrobial activities of the melanin were studied.

Materials and Methods

Screening and Isolation of Melanin Producer Strains

The marine green alga *Ulva lactuca* (Kingdom: *Plantae*, Phylum: *Chlorophyta*, Class: *Ulvophyceae*, Order: *Ulvales*, Family: *Ulvaceae*, Genus: *Ulva* [22]) was collected from a beach in the North West of the Atlantic coast of Morocco. The collected green alga was thoroughly washed thrice with sterile distilled water. The alga sample was crushed in sterilized glass mortar and the extract was resuspended in physiological water then serial dilutions were spread over LB agar (Tryptone 20 g/l, Yeast extract 5 g/l, Sodium chloride 10 g/l, Agar 15 g/l). Different isolates were selected and purified to be further used for the screening step over the M9 minimal medium (Na_2HPO_4.12 H_2O, 15.02 g/l ; KH_2PO_4, 3 g/l ; NH_4Cl, 1 g/l ; NaCl, 0.5 g/l ; $MgSO_4$, 1 mM ; $CaCl_2$, 0.1 mM ; Agar, 20 g/l ; pH 7.4), and containing L-tyrosine at 1 g/l, and trace elements of $CuSO_4$ and $FeSO_4$ at 10 µM each.

Scanning Electron Microscopy (SEM)

Following growth in LB liquid medium at 37°C, cells were harvested during the logarithmic phase by centrifugation (10000 rpm, 10 min, 4°C) then were fixed by glutaraldehyde after several washing steps using water and ethanol [23], and observed under scanning electron microscope (SEM) QUANTA 200 with a high magnification of 13,000X.

Molecular Identification by PCR and Sequencing of 16S rDNA

The genomic DNA of isolate was extracted according to the following method. Isolate was grown in 10 ml LB medium under shaking at 30°C for 48 h. A volume of 2 ml of this culture was harvested at 8000 rpm for 5 minutes at 4°C. The pellet was then washed once with TE buffer (10 mM Tris-HCl/1 mM EDTA (pH7.7)) and resuspended in 500 µl of TE buffer. Then, the sample was heated in boiling water for 10 min, allowed to cool in ice bath for 5 min and centrifuged at 8000 rpm for 3 min. The supernatant (300 µl) was recovered, transferred to a new tube and stored at +4°C until use [24].

The universal eubacterial primer 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3') were used [25] to amplify 16S rDNA on a thermal cycler (Techne Genius, Cambridge, UK). The amplification reaction was performed in a final volume of 20 µl containing 0.5 µM of each primer, 200 µM each dNTP, 0.2 units *Taq* DNA polymerase, 1.5 mM $MgCl_2$ and 2 µl of DNA sample in 1x *Taq* polymerase buffer. The mixture was first denatured at 94°C for 2 min. Then, 35 cycles of PCR were performed by denaturation at 94°C for 30 sec, primers annealing at 55°C for 30 s and primer extension at 72°C for 1 min 30 sec. At the end of the last cycle, the mixture was incubated at 72°C for 5 min. For each reaction, a negative control missing DNA template was included. Efficient amplification was confirmed by gel electrophoresis on 1% agarose gel. PCR products were purified and sequenced using an ABI Prism sequencing apparatus (ABI Prism 3130 Genetic Analyzer, Applied Biosystems) and data analysis was carried out using Chromas Pro software.

Growth of Cells and Production of Melanin

Cells were grown overnight in LB liquid medium at 37°C under agitation (140 rpm), then 1 ml of cells suspension was inoculated in 100 ml of M9 liquid medium supplemented with 1 g/l Yeast Extract and 1g/l L-tyrosine.

Production of melanin by *Pseudomonas balearica* U7 strain was carried out at 30°C and at 140 rpm. Samples of cultures were taken in intervals for quantitative and qualitative analysis. Their optical densities $OD_{600\ nm}$ were measured in a spectrophotometer (UV-VIS SELECTA, EU). Spectrophotometric determination at 400 nm of pigmented supernatants (6000 rpm, 5 min) was used for melanin quantification according to a calibration curve with synthetic melanin (Sigma-Aldrich, St. Louis, MO, USA) [26].

The kinetic of L-tyrosine uptake during the fermentation was determined according to the method of Arnow [27]. Briefly, 1ml of sulfate mercuric solution (15% in 5N sulphuric acid) was added to 1 ml of cell-free supernatant and left at 95°C for 10 minutes. Then, 1 ml of nitrite reagent (0,2% of sodium nitrite) was subsequently added and the total volume was made up to 5 ml with distilled water.

Melanin Crude Extraction

After incubation, the medium was centrifuged at 8000 rpm for 15 min to separate the supernatant and cells. The extraction process of pigment is similar to the melanin purification protocols reported in previous studies [5,28] with slight adaptations: Firstly, the pH of the supernatant was adjusted to 10 with 5M NaOH to ensure complete polymerization of melanin, then to pH 2 with 5M HCl, the mixture was centrifuged at 8000 rpm for 20 min to obtain the melanin crude extract. Secondly, equal volume of chloroform, ethyl acetate and methanol (1V:1V:1V) were added and mixed, to remove proteins and lipids, this step was repeated 2 to 3 times. Finally, aqueous phase was concentrated by a rotary evaporator and the pigment was then collected [29].

Chemical Analysis of Crude Pigment Extract

The chemical analysis of pigment crude extract was carried out using the modified method of Fava, et al [30]. The solubility of the pigment was assessed in different solvents. Reactions with oxidizing agents such $KMnO_4$ 1%, and H_2O_2 were determined. Reducing agents such as $Na_2S_2O_4$ 5% were also tested for reaction with the pigment. Assay for phenol containing molecules was used by reaction of the obtained pigment with 1% $FeCl_3$ (v/w).

UV-Visible Spectroscopic Analysis

V-visible spectrum scanning of the obtained melanin was performed in a solution of Tris-HCl 50 mM (pH 8.5) to a concentration of 0.01 g/l, and was scanned from 200 to 1100 nm wavelengths. Standard synthetic melanin was used as standard and Tris-HCl 50 mM (pH 8.5) as blank. The spectra were determined using UV-Visible spectrophotometer (Jasco V-350, USA).

Fourier Transform Infrared Spectroscopy (FTIR)

The extracted pigment was ground with IR grade KBr (1:10). The FTIR spectrum was recorded at 4000-400 cm^{-1} using a VERTEX 70 FT-IR spectrophotometer.

^{1}H NMR

The samples were prepared by dissolving 50 mg of U7 melanin and synthetic melanin in 3.5 ml of a mixture of deuterium oxide/

ammonia. The mixture was obtained by adding 0.01 ml of aqueous ammonia (33%) to 10 ml of deuterium oxide to reach pH 10; this treatment ensures the total solubilization of melanin [31]. NMR spectra were recorded on a BRUKER AC 200 apparatus.

Antimicrobial Activity of Extracted Pigment

Test microorganisms, including *Staphylococcus aureus, Erwinia chrysanthemi 3937VIII, Erwinia carotovora 197 stp*^{R}*, Echerichia coli* TG1 and *Candida albicans,* were separately cultivated on the Muller -Hinton agar plates; swab inoculations of the microbes [Table-1] were made on the surface to produce a lawn culture. Antimicrobial activity of 10 µg of pigment extract was tested by well diffusion method [29]. The plates were incubated at 30°C or 37°C for 24 hrs. and checked out for halo zone apparition. Presence of halo zone around the well indicated a positive antimicrobial property. Three experiments were independently performed.

Table 1- List of bacteria and fungus used in the antimicrobial test

Strains used	Incubation at	Provenance
Staphylococcus aureus ATCC 9144	37°C	
Candida albicans ATCC 10223	30°C	
Escherichia coli TG1	37°C	CNRS-LB Marseille, France
Erwinia chrysanthemi 3937VIII	30°C	
Erwinia carotovora 197 stp^R	30°C	

Antioxidant Activity of the Extracted Pigment

DPPH• radical scavenging activity of the produced melanin was tested according to the developed method of Anissi, et al [32]. Briefly, quantities from 0.1 mg to 5 mg of melanin in a volume of 1.5 ml were mixed with the same volume of a 0.4 mmol/l DPPH•, using a JASCO SFS-492 series stopped-flow systems apparatus connected to a Jasco J-815 Series spectrophotometer (Jasco, USA). Results were displayed using Spectra Manager II software (Jasco, USA). The decrease in the absorbance for the DPPH• radicals was followed at 515 nm ($\varepsilon_{515\,nm}$ = 10870 (mol/l)$^{-1}$.cm^{-1} in methanol). Absolute methanol solution replaced the sample solution as blank control.

The antioxidant activity of melanin was expressed as *IC100*. The *IC100* value was defined as the amount (in µg/ml) of melanin needed to scavenge 100% of the initial DPPH• concentration, and *n* the ratio of the initial DPPH• concentration to *IC100* [32].

Results

Strain Selection

Among the different bacteria isolated from the alga material, 11 isolates were able to grow on solid and liquid M9 media supplemented with L-tyrosine, but only five isolates were capable to secrete a black/brown pigment. One of these isolates, designated U7, was determined as producing large amount of a brown pigment in a relatively short time period in both solid and liquid M9 medium supplemented with L-tyrosine and yeast extract (YE).

Strain Characterization

U7 strain was identified based on morphological, physiological and biochemical characteristics. The sequence of 16S rDNA was submitted to NCBI GenBank database (Accession number KJ940973), it was obtained with size of about 1457 pb and showed a GC content of 54.2%. Comparison of this sequence to the GenBank using the program BLAST and the phylogenetic analysis [Fig-1] with the reference 16S rDNAs revealed a high similarity (99% homology) with *P. balearica OAct420 (*accession number KC514126) and *P. stutzeri* LS401 (accession number U26417). The use of molecular approach and biochemical tests has led to a more refined identification of the isolate U7 as *P. balearica* with regards to some shared physiological traits such as the growth at 44°C, xylose assimilation and tolerance of high concentrations of sodium chloride up to 1,5 M as was shown by Bennasar, et al [33]. SEM micrograph of U7 strain is shown in [Fig-2], the high magnification SEM image of the isolate U7 show miniscule rod-shaped cells.

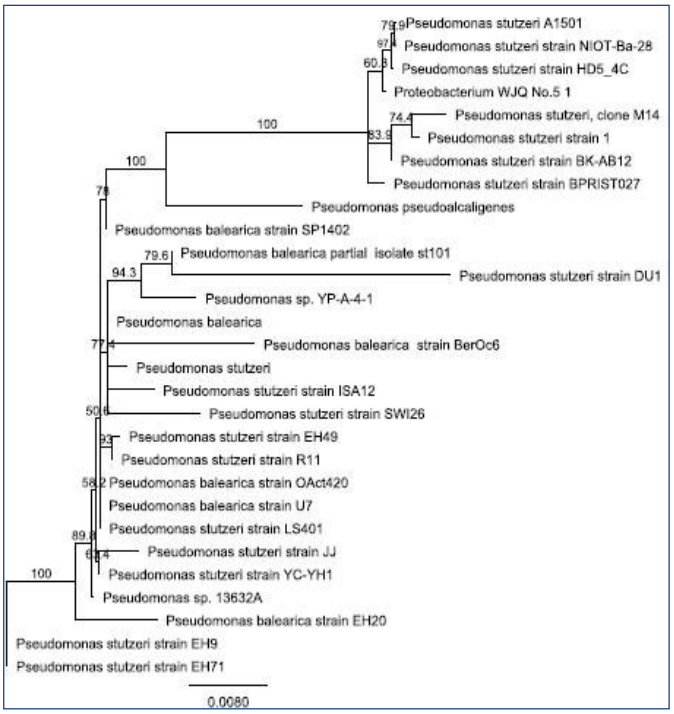

Fig. 1- Phylogenetic tree of *Pseudomonas balearica* strain U7

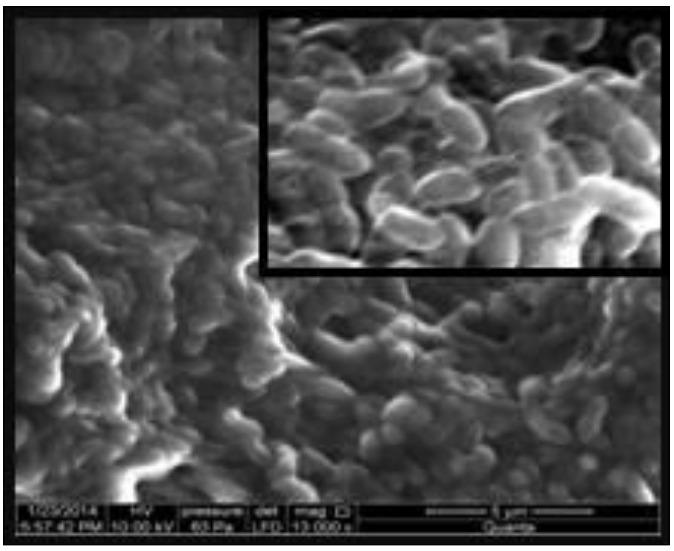

Fig. 2- Observation by SEM of the strain U7 (mag. 13000×)

Growth of U7 Bacterium Strain and Production of Melanin

A volume of 1 ml from an overnight culture of the isolate U7 in LB, with an optical density of 1.116, was inoculated into 250 ml conical flasks, each containing 100 ml of the production medium. Culture was carried out under orbital shaking at 30°C for 5 days. Superna-

tant was taken to determine the growth, melanin production and L-tyrosine depletion.

[Fig-3] shows the effect of the presence of both, yeast extract and L-tyrosine on the production of melanin pigment in a minimal culture medium by the isolate U7. Results showed that the bacterium use L-tyrosine as substrate to produce melanin under specific physical and chemical conditions. However, the addition of yeast extract enhanced the production of melanin to reach values up to 110 mg/l; versus 29 mg/l with the presence of L-tyrosine alone.

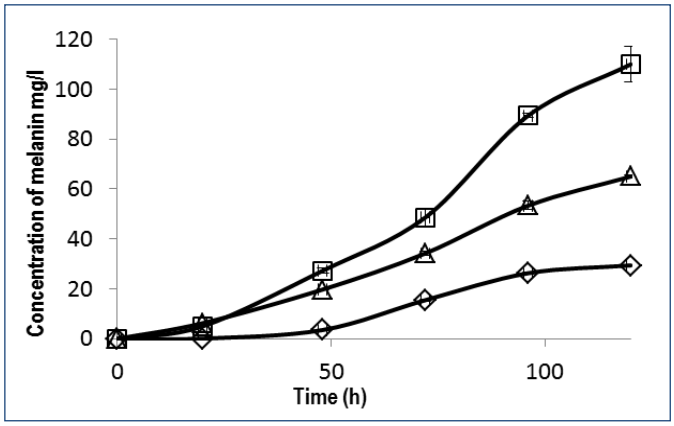

Fig. 3- Evolution of the concentration of the melanin in the cell-free medium as function of time in M9 medium containing YE: Yeast extract 1g/l ◊, TY: Tyrosine1g/l Δ, and TY/YE: Tyrosine and Yeast extract 1g/l each ð. Values are reported as mean ± SD

[Fig-4] shows the kinetic of the depletion of L-tyrosine and the formation of melanin, altogether with kinetic of growth of the isolate U7. Results showed that the production of melanin started during the early phase of growth to reach its maximum during the late phase of growth (110 mg/l). The production of melanin showed a delay compared to the uptake of L-tyrosine by the biocatalyst U7. During the early stationary phase of incubation (72 hrs.), the uptake of L-tyrosine reaches 0.935 g/l of the initial amount, showing that the tyrosine was converted to other metabolites before its polymerization into melanin; therefore melanin is most probably produced by polymerization of other phenolic and indolic metabolites derivatives from tyrosine metabolism.

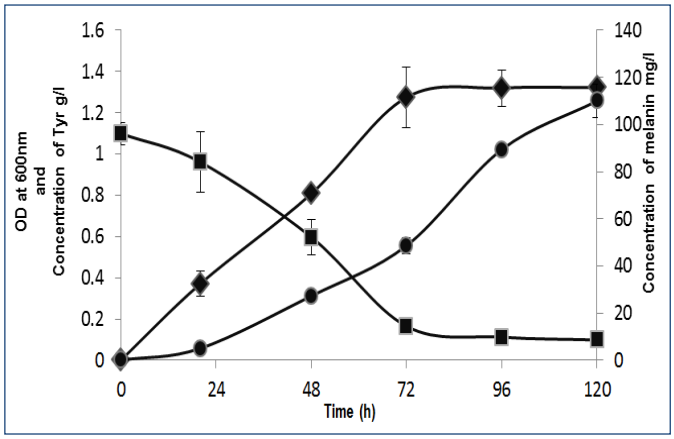

Fig. 4- Growth curve of U7 strain (♦), depletion of tyrosine (■) and formation of melanin (●), as function of time. Values are reported as mean ± SD

The effect of different temperatures (20°C to 45°C) and pH of the medium (pH 5.0 to 9.0) on melanin production was studied [Fig-5]. Results showed that optimal temperature for growth and melanin production was determined in the range of 30 to 37°C. At alkaline conditions (7.0 <pH<8.2), the production of melanin took higher values, ranging from 92.42 mg/l to 112.8 mg/l, Moreover, the pH of the cell-free supernatant increased as the bacterium growth progresses to stabilize at pH 8.2, at that pH value the maximum production of melanin occurred.

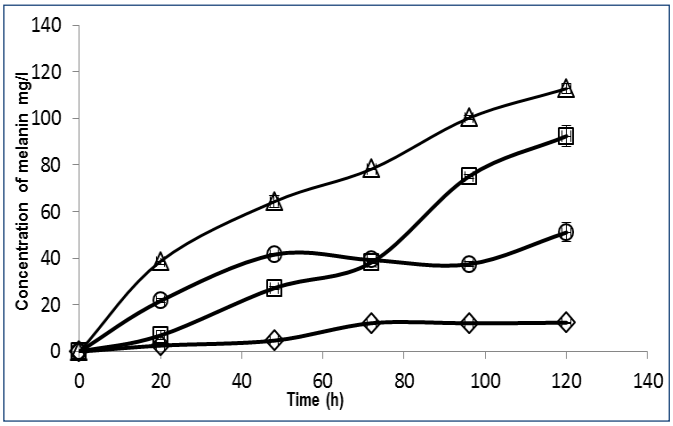

Fig. 5- Effect of pH on the performances of the strain U7 for the production of melanin. pH 6 (◊), pH 7 (ð), pH 8(Δ) and pH 9 (○).Values are reported as mean ± SD

Pigment Extraction and Characterization

The dark brown pigment from the cell-free medium was extracted with a concentration of 110 mg/l. the extracted pigment was subjected to chemical tests and showed the results presented in [Table -2]. We found that the pigment produced by the isolate U7 meets the criteria as obtained for melanin isolated from other bacteria such as *Aeromonas media* [34], *Escherichia coli* [35] and *Klebsiella sp. GSK* [7].

Table 2- Physical and chemical properties of melanin produced by the strain U7

Tests	Results
Solubility in :	
Water hot/cold	Soluble
HCl 1N	Insoluble
NaOH 1N	Soluble
KOH 1N	Soluble
Methanol	Sparingly soluble
Ethanol	sparingly soluble
Chloroform	Insoluble
Acetone	Insoluble
Hexane	Insoluble
Ethyl acetate	Insoluble
Acetic acid	Insoluble
Ether	Insoluble
DMSO	Insoluble
Addition of oxidizing agents:	
H_2O_2	Decolorization
$Na_2S_2O_4$ 5%	weak Decolorization
$KMnO_4$ 1%	Absence of decolorization
Test of polyphenols with $FeCl_3$ 1%	Floculation of a brown precipitate

Spectroscopic Analysis of the Pigment

The UV / Visible spectrum of the pigment, produced by the strain U7, showed strong absorbance in the UV region [Fig-6]. The absorbance maxima were in the range of 280-335 nm and progressively decreased in the visible wavelengths as for the typical absorption profile of synthetic melanin [36,37].

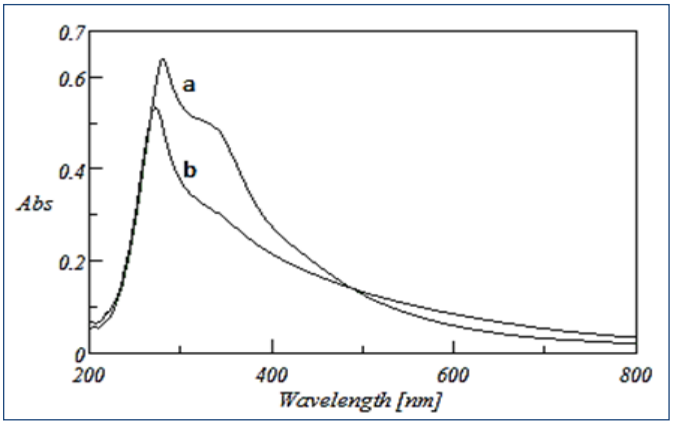

Fig. 6- UV-visible spectra of the obtained melanin **(a)** and synthetic melanin **(b)**

FTIR spectral study was conducted to get information about melanin functional groups and structure [Fig-7]. The FTIR spectra of synthetic melanin and melanin produced by strain U7 showed common signals. A broad band from 3365-2880 cm^{-1}, attributed to the stretching vibrations of OH and NH groups, and containing also the signal attributed to stretching vibration of aliphatic C-H. Resemblance in the main absorption peaks, notably in the main absorption band centred near 3300 cm^{-1}, which reveals the presence of the -OH group bonding with the -NH group. However, the broad band with a pick at 3300 cm^{-1} does not show a centred signal compared to the one from the synthetic melanin. The band centred at 1630 cm^{-1} is attributed to vibrations of aromatic ring C=C bounds and/or of aromatic conjugated C=O groups. More absorption peaks were observed in FTIR spectrum of bacterial U7 pigment, the one centred at 1447 cm^{-1} must correspond to aliphatic C-H bending and/or C-N stretch, and/or the carboxylate ion groups. The other little peaks may be ascribed to alkene C-H substitution in the melanin pigment. These absorptions are common and characteristic for melanin from several biological materials as described in earlier reports [7,38]. Based on the Raper-Mason pathway for melanogenesis [39], different structures of potential building blocks were taken in consideration in order to characterize the structure of the produced melanin by the strain U7.

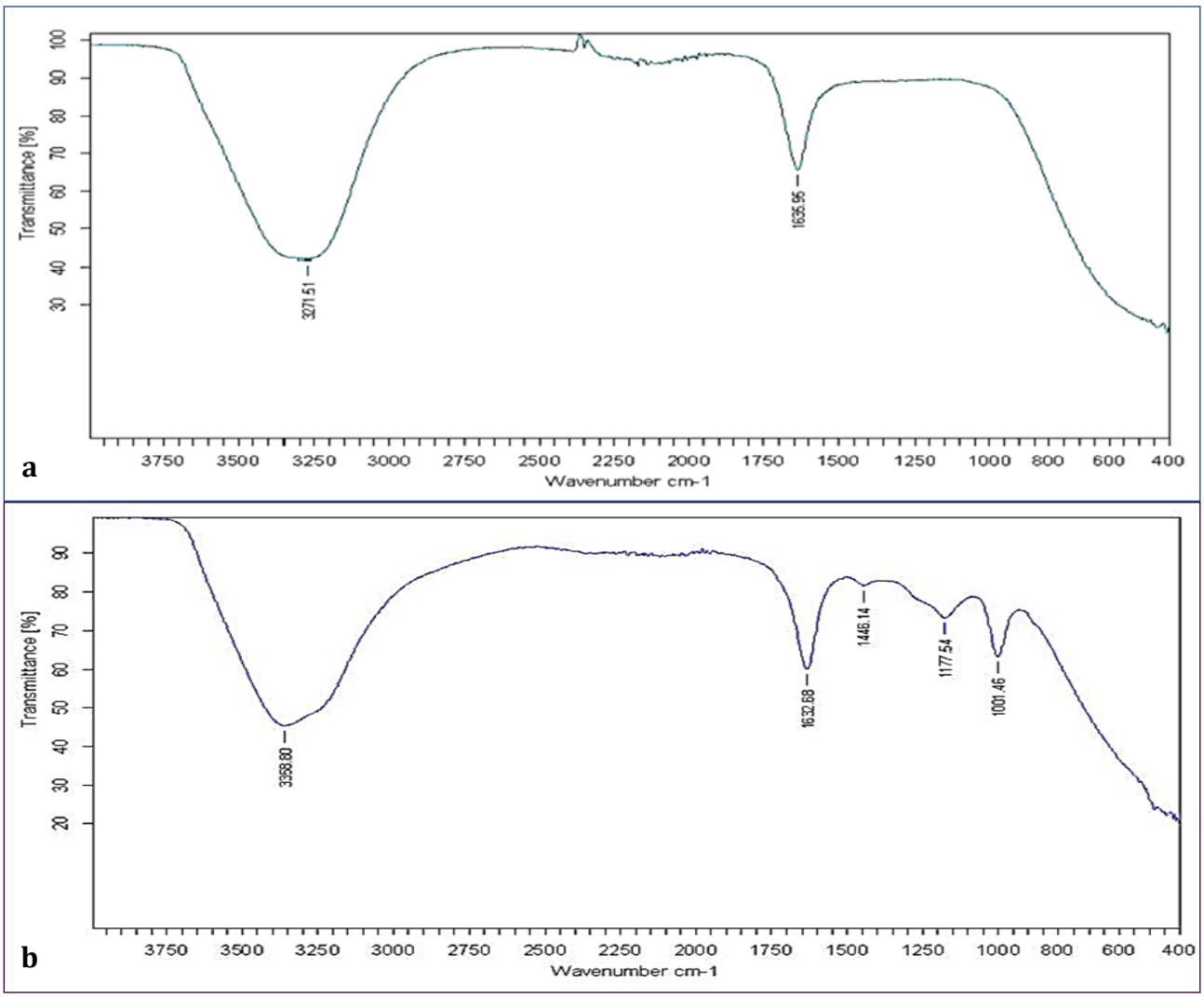

Fig. 7- FTIR spectra of synthetic melanin **(a)** and bacterial melanin produced by strain U7 **(b)**

The ^{1}H NMR spectrum of melanin from the isolate U7 showed signals spread out over the aliphatic and aromatic regions (data not shown). The signal at δ ppm of 0.857 has been ascribed to methyl groups of alkyl fragments, such as CH_2CH_3. In addition, signals in the absorption region from 3.10 to 4.20 ppm can be assigned to protons on carbons attached to nitrogen and/or oxygen atoms. Peaks between 7.48 and 7.25 can be attributed to protons attached to not yet substituted aromatic or heteroaromatic rings.

Antimicrobial Activity of Melanin Extract

The melanin pigment showed considerable antimicrobial activity against five different microorganisms, and results are presented in [Table-3]. The results showed strong anti-bacterial activity against *S. aureus*, followed by *E. coli*, *C. albicans*, and the phytopathogenic strains *E. chrysanthemi and E. carotovora*. The diameter of inhibition zone (DI) was higher against *S. aureus* (DI = 41 mm) than the inhibition zone produced against *E. coli* (DI = 30 mm), phytopathogenic strains *Erwinia carotovora* (DI = 30 mm), *Erwinia chrysanthemi* (DI = 28 mm), and *Candida albicans* (DI =23 mm).

Table 3- Antibacterial activity of bacterial melanin produced by strain U7 against pathogenic strains

Pathogenic strain	Diameter of the inhibition (mm)
E. coli TG1	33 ± 2
Staphylococus aureus	41 ± 2
Candida albicans	23 ± 1
Erwinia chrysanthemi 3937VIII	28 ± 3
Erwinia carotovora 197 Stp^R	30 ± 2

DPPH· Scavenging Activity

Free radical scavenging activity of U7 melanin was evaluated by performing DPPH· assay. With an increase in U7 melanin concentration, the DPPH· scavenging increased gradually (data not shown). Melanin chemical structure encloses functional groups such as COOH, OH and NH_2 which could capture electrons and scavenge Reactive Oxygen Species (ROS). Melanin exhibited second-order kinetics for its reaction with DPPH·. Variations of k'_2 values as a function of time showed the occurrence of a maximum (k'_{2max}), as shown in [Fig-8](a) and these subsequently showed a linear correlation when plotted as a function of the initial melanin concentration, as presented in [Fig-8](b). IC_{100} and n were 6.23 ± 0.36 g/l and 3.68± 0.41, respectively. The slope of the reciprocal for the DPPH· concentration as a function of time, extracted from the linear curve in [Fig-8](a), showed a linear dependence with the initial concentration of melanin [Fig-8](b). In contrast to ascorbic acid (IC_{100} of 95.400 ± 0.005 µmol/L and n of 2.157 ± 0.168), melanin showed a slow kinetic and a high IC_{100} values.

Discussion

In this study, we report the isolation of a from a *Pseudomonas balearica* strain U7 from the marine alga *Ulva lactuca*, which produces high level of melanin using L-tyrosine as precursor in a minimal medium. The maximum concentration is110 mg/l after 72h of growth; suggesting that, like many other melanin-producing *Pseudomonas* strains, *P. balearica* U7 strain possesses among others a monophenol hydroxylase activity [40,41]. We have demonstrated that the pigment produced by strain *Pseudomonas balearica* is melanin as confirmed by FTIR, ^{1}H-NMR and UV-Vis spectroscopic analysis and meets the criteria as obtained for melanin isolated from other bacteria [7,34,35]. However, the particularity of melanin from the isolated *Pseudomonas balearica* strain U7 was its water-solubility under slightly alkaline conditions, this may be due to the abundance of carboxyl groups (COOH) present in the molecule, and/or the high degree of amino groups incorporated into the melanin.

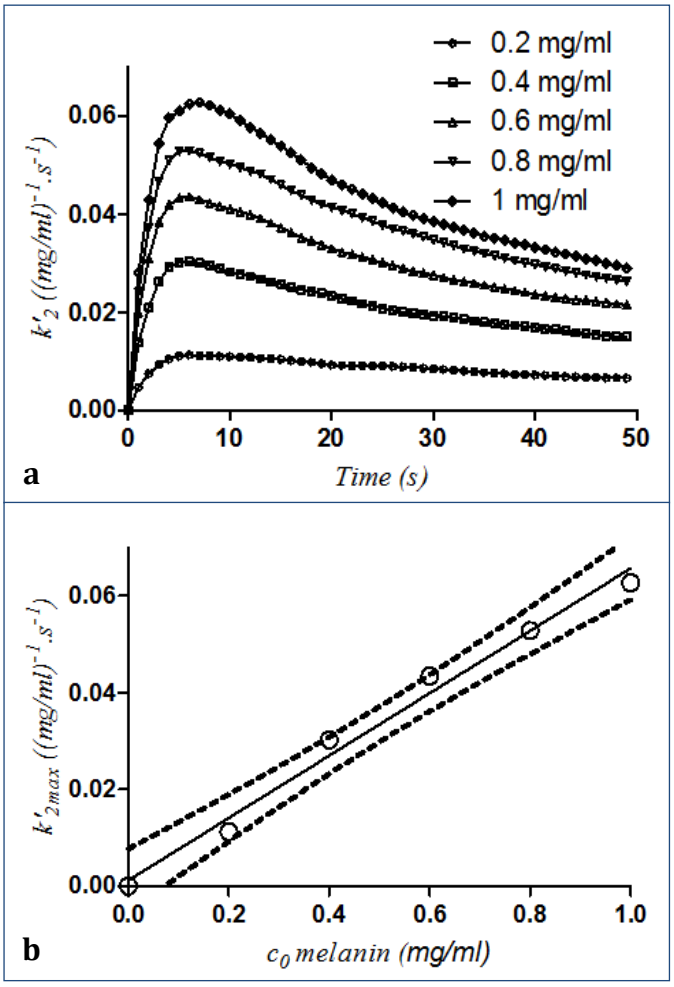

Fig. 8- Variations of k'_2 as function of time **(a)** and dependence k'_{2max} on the initial concentration melanin **(b)**

A source of water-soluble melanin is highly desirable, since the water-insoluble melanin requires severe treatments such as boiling in strong alkali, or the use of strong oxidants such as hydrogen peroxide, which often damage the melanin and restricts its use [42].

The strain U7 has a high capacity to produce melanin even in LB medium without further supplementation with L-tyrosine (data not shown), suggesting that pathways other than tyrosine metabolism might be involved in bacterial melanin production.

Melanin extracted from *P. balearica* was characterized for its antimicrobial activity against some Gram-positive and Gram-negative bacteria. Vasanthabharathi [26] reported that melanin produced by actinomycetes can exhibit a maximum of 22 mm of inhibition diameter against *E. coli*. Yet, Vallimayil [40] recorded an inhibition diameter of 25 mm against *E. coli* using melanin produced by *Pleurotus djamor*. To our knowledge, this is the first time when the antibacterial activity of melanin was tested against the phytopathogenic bacteria *E. chrysanthemi and E. carotovora,* these promising results should be examined for a potential use of our strain as biocontrol agent in the near future. Screening of new microbial metabolites

that have antioxidant activities has been the major area of focus of many researchers in pharmaceutical and food industries [3]. Melanin could capture electrons and scavenge ROS (Reactive Oxygen Species) produced through organisms metabolism or due to UV radiations and protect from oxidative stress [44,45]. In this study the melanin pigment extracted of *P. Balearica* possesses efficient free radical scavenging activity of a model DPPH radical. These properties indicate high potential of the strain U7 to be used as an industrial biocatalyst for the melanin production.

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Conflict of Interest: No conflict of interest is declared.

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