Biosynthesis of catechol melanin from glycerol employing metabolically engineered *Escherichia coli*

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**Abstract**

**Background:** Melanins comprise a chemically-diverse group of polymeric pigments whose function is related to protection against physical and chemical stress factors. These polymers have current and potential applications in the chemical, medical, electronics and materials industries. The biotechnological production of melanins offers the possibility of obtaining these pigments in pure form and relatively low cost. In this study, *Escherichia coli* strains were engineered to evaluate the production of melanin from supplemented catechol or from glycerol-derived catechol produced by an *Escherichia coli* strain generated by metabolic engineering.

**Results:** It was determined that an improved mutant version of the tyrosinase from *Rhizobium etli* (MutmelA), could employ catechol as a substrate to generate melanin. Strain *E. coli* W3110 expressing *MutmelA* was grown in bioreactor batch cultures with catechol supplemented in the medium. Under these conditions, 0.29 g/L of catechol melanin were produced. A strain with the capacity to synthesize catechol melanin from a simple carbon source was generated by integrating the gene *MutmelA* into the chromosome of *E. coli* W3110 trpD9923, that has been modified to produce catechol by the expression of genes encoding a feedback inhibition resistant version of 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase, transketolase and anthranilate 1,2-dioxygenase from *Pseudomonas aeruginosa* PAO1. In batch cultures with this strain employing complex medium with 40 g/L glycerol as a carbon source, 1.21 g/L of catechol melanin were produced. The melanin was analysed by employing Fourier transform infrared spectroscopy, revealing the expected characteristics for a catechol-derived polymer.

**Conclusions:** This constitutes the first report of an engineered *E. coli* strain and a fermentation process for producing a catechol melanin from a simple carbon source (glycerol) at gram level, opening the possibility of generating a large quantity of this polymer for its detailed characterization and the development of novel applications.

**Keywords:** Metabolic engineering, Melanin, Catechol, Tyrosinase, *Escherichia coli*

**Background**

Melanins are polymeric pigments found in most biological groups [1]. These compounds have diverse functions, mostly related to stress protection from physical and chemical factors. Melanins result from the oxidative polymerization of phenolic and indolic compounds [2]. Monomer precursors for the various types of natural melanins include l-tyrosine, l-3,4-dihydroxyphenylalanine, homogentisic acid, 1,8-dihydroxynaphthalene, glutaminyl-3,4-dihydroxybenzene and catechol [3, 4]. The first step in the synthesis of melanins involves the enzyme-catalysed oxidation of the precursor monomers into their respective quinones, which undergo further spontaneous oxidation to generate the high molecular weight melanins. Tyrosinase and laccase have been identified as the enzymes involved in melanogenesis in various microbial species. These are copper-containing enzymes.
oxidoreductases, which can employ as substrates various mono- and polyphenolic compounds [5, 6].

Diverse studies have determined potential industrial applications for melanins. These polymers can act as UV absorbers, scavengers of free radicals and reactive oxygen species, amorphous semiconductors, cation exchangers, X and γ-ray absorbers [2, 7, 8]. Melanins can also serve as a template for the synthesis of silver and gold nanostructures with potential uses in the medical and food industries [9, 10]. Considering the physicochemical complexity and diversity of melanins, it is expected that further industrial applications will be found in the near future.

There is considerable interest in the development of production processes for obtaining melanins with high purity and at a relatively low cost. The biotechnological production of melanin derived from L-tyrosine (eumelanin) has been reported employing recombinant Escherichia coli strains. This has been achieved by the heterologous expression of the gene encoding the enzyme tyrosinase from the bacteria Rhizobium etli or Streptomyces antibioticus [11–13]. The production processes with these recombinant microorganisms are based on the conversion of L-tyrosine that is supplemented to the culture medium, into melanin in liquid cultures. In a recent development, an E. coli strain was modified by metabolic engineering to transform glucose to melanin by increasing its capacity to synthesize the precursor L-tyrosine and simultaneously expressing the gene encoding tyrosinase from R. etli [14].

Most efforts directed towards the biotechnological production of melanins have been directed to eumelanin. This is motivated in part because humans produce eumelanin. However, other types of melanins have physicochemical properties different from those of eumelanin and thus have particular potential applications. The allomelansins are polymers derived from catechol or other substrates. A recent study has demonstrated that a melanin synthesized by the basidiomycetous fungus Rhizoctonia solani is derived from catechol [15]. In another report, the production of catechol melanin by the obligate aerobic nitrogen-fixing bacterium Azotobacter chroococcum was demonstrated [16]. These organisms could potentially be employed as producers of catechol melanin in a biotechnological process. However, important challenges to overcome would be related to defining the optimal growth and catechol melanin production conditions. An alternative to this approach could be to employ an organism that can be easily grown in a bioreactor under laboratory conditions and genetically modify it to synthesize melanin. In this report, we used the bacterium E. coli as a platform to generate strains with the capacity to synthesize catechol melanin. As an initial approach, the MutmelA gene from R. etli was expressed in E. coli W3110 to construct a strain for converting catechol supplemented in the culture medium to catechol melanin. To improve this production system, we employed E. coli strain W3110 trpD9923, a mutant that overproduces anthranilate, which was modified to produce catechol from glucose [17]. This strain was further engineered by the chromosomal insertion of gene MutmelA from R. etli (Fig. 1). The resultant strain was grown in liquid fed batch cultures in complex medium with glycerol as carbon source, where 1.21 g/L of catechol melanin were produced in 55 h. The chemical identity of the produced catechol melanin was determined by employing Fourier transform infrared spectroscopy.

**Methods**

**Bacterial strains and plasmids**

The E. coli strains and plasmids employed in this study are described in Table 1. E. coli W3110 is a wild type strain, used as a parental host [18]. Strain W3110 trpD9923 is a mutant in the trpD gene generated with ultraviolet radiation, it accumulates anthranilate, and it is an L-tryptophan auxotroph [19]. Plasmid pTrcMutmelA carries the MutmelA gene that encodes a tyrosinase from R. etli CFN42 [12]. MutMelA is a mutant version of the MelA tyrosinase, having a single nucleotide change that resulted in the replacement of Asp535 by Gly. It has been determined that melanin production occurs earlier in cultures with E. coli strains expressing MutmelA when compared to strains expressing wild-type MelA, but melanin rate of synthesis is similar to either version of the enzyme [13]. Plasmid pTrc-ant3 carries the antABC genes coding for the anthranilate 1,2-dioxygenase from P. aeruginosa PAO1 [17]. Plasmid pILBaroGbrtktA carries the araGbr and tktA genes from E. coli encoding a feedback inhibition resistant version of 3-deoxy-d-arabinohexulosonate 7-phosphate synthase and transketolase, respectively [20].

**Construction of pLoxGentrcMutmelA and chromosomal integration of MutmelA in E. coli W3110 trpD9923**

A 1830 bp DNA fragment containing gene MutmelA, was obtained by digesting plasmid pTrcMutmelA with restriction enzymes NcoI and Smal and ligated to plasmid pLoxGentrc digested with the same restriction enzymes [21]. The resulting plasmid was named pLoxGentrcMutmelA. Plasmid pLoxGentrcMutmelA was employed as a PCR template to amplify a 3761 bp product that includes the MutmelA gene under transcriptional control of the IPTG-inducible trc promoter [21]. Primers employed were trcmelAlacZrvs and trcmelAlacZfwd which include 45 bases with homology to the 5’and 3’ terminal regions of lacZ, respectively [21]. The PCR reaction was
performed using standard buffer conditions, Kapa HiFi DNA polymerase (Kapa BioSystems), one cycle of initial denaturation at 94 °C for 3 min, 30 cycles with denaturing at 94 °C for 30 s, annealing at 56 °C for 60 s and extension at 68 °C for 60 s/kb in a total volume of 50 μL. The PCR product was gel-purified and digested with restriction enzyme DraI to eliminate residual plasmid pLoxGen. The PCR product was gel-purified and digested with restriction enzyme DraI to eliminate residual plasmid pLoxGen. The PCR product was gel-purified and digested with restriction enzyme DraI to eliminate residual plasmid pLoxGen.

**Table 1. E. coli strains and plasmids used in this study**

| Strains          | Relevant features                                           | Source               |
|------------------|-------------------------------------------------------------|----------------------|
| Strains W3110    | F−,λ−, Inv (rmd-mem)                                        | ATCC27325            |
| W3110 trpD9923   | W3110 [F− Δ− Inv (rmd-mem)] 1 trpD9923 tryptophan auxotroph | [19]                 |
| W3110 trpD9923 MutmelA | W3110 trpD9923 with R. etli MutmelA gene inserted in the chromosomal lacZ locus | This work            |
| Plasmids         |                                                             |                      |
| pTrcMutmelA      | Gene MutmelA cloned in expression vector pTrc99A             | [12]                 |
| pTrc-ant3        | Carries the antABC genes from P. aeruginosa PAO1 under control of the trc promoter from pTrc99A, ampicillin resistance | [17]                 |
| pLBaroGbrtktA    | Carries the E. coli tktA gene with its native promoter and the aroGbr gene under control of the lacUV5 promoter, tetracycline resistance, pACYC184 origin of replication. Expression-chromosomal integration vector containing the trc promoter, a multiple cloning site, the T1 and T2 rmb terminator sequences and the lacI gene | [20] [21]            |
| pLoxGentrctrMelA | Derivative of pLoxGentr containing gene MutmelA from R. etli | This work            |
model ADI 1010 (Applikon, The Netherlands). Bioreactor cultures were started from a strain sample taken from a frozen stock that was placed in tubes with 4 mL of LB with the required antibiotics at 37 °C and 150 rpm agitation. After 12 h, the total volume of the previous culture was placed in a 250 mL flask with 75 mL of LB medium with the required antibiotics for each strain and incubated at 37 °C and 150 rpm. After approximately 12 h, the cultures reached an optical density at 600 nm (OD\textsubscript{600}) of 4.0, and an aliquot was taken to inoculate the bioreactor at a starting OD\textsubscript{600} of 0.2. For bioconversion experiments, a bioreactor with 800 mL of LB supplemented with 20 µg/mL CuSO\textsubscript{4}, 0.1 mM IPTG and Ap 200 µg/mL was inoculated at a starting OD\textsubscript{600} of 0.2. When the culture reached an OD\textsubscript{600} of approximately 6.0, the contents of the bioreactor were transferred to bottles under sterile conditions and centrifuged at 4000 rpm for 4 min. The cell pellet was resuspended in modified M9 minimal salts medium supplemented with 20 g/L of glycerol, 0.3 g/L of catechol, 20 µg/mL CuSO\textsubscript{4}, 0.1 mM IPTG and Ap 200 µg/mL. For all bioreactor experiments, air flow rate was maintained at 1 vvm and agitation varied from 500 to 700 rpm to maintain the level of dissolved oxygen above 20 %. Dissolved oxygen was measured with a polarographic oxygen probe (AppliSens; Applikon, Inc., Foster City, CA, USA). Culture temperature was maintained at 30 °C and pH at 7.0. Catechol melanin was recovered from the culture medium by adjusting the pH to 2.0 with HCl 6 N and maintaining it at 4 °C for 16 h. Afterwards, the culture medium was centrifuged for 6 min ant 8000 rpm. The remaining supernatant was left for another 16 h and centrifuged. The precipitated catechol melanin was washed with distilled water and dried at 45 °C for 24 h.

**Analytical methods**

Dry cell weight (DCW) was calculated multiplying the 600 nm absorbance by a previously determined coefficient factor of 0.37 g/L [13]. Samples taken during the cultivation period were centrifuged at 10,000 rpm for 2 min at room temperature. The supernatant was filtered using a 0.45 µm syringe filter and stored at −20 °C for subsequent analysis. Glycerol and acetate were determined by high performance liquid chromatography (HPLC) (Waters, Milford, MA, USA), using an Aminex HPX-87H column (300 × 7.8 mm; Bio-Rad, Hercules, CA, USA). Running conditions were 5 mM H\textsubscript{2}SO\textsubscript{4} as mobile phase, a flow of 0.5 mL/min and temperature of 50 °C. Glycerol detection was performed by refraction index detection and acetate by photodiode array at 210 nm. Catechol and anthranilate were determined from the culture medium and not from biomass since the former is the fraction available for recovery. The method employed was HPLC (Agilent Technologies, Palo Alto, CA, USA) using a Synergy Hydro C18 4 µm column (4.6 × 150 mm, Phenomenex, Torrance, CA, USA); running conditions were 0.1 % trifluoroacetic acid in 20 % methanol as mobile phase, a flow of 0.5 mL/min. Detection was performed by photodiode array at 330 nm for anthranilate and 280 nm for catechol. Melanin samples employed for Fourier transform infrared spectroscopy (FTIR) analyses were obtained from the bioreactor cultures described in this article. FTIR spectra were performed at room temperature using a Bruker Tensor 37 spectrometer with a Platinum ATR Accessory by utilizing a resolution of 1 cm\textsuperscript{−1} and 32 scans per spectrum. The samples were finely ground and placed into the sampling area while getting in touch with the diamond ATR-crystal under pressure application.

**Results and discussion**

**Bioconversion production of catechol melanin by an E. coli strain expressing MutMelA**

During characterization of an E. coli strain that expressed enzyme MutMelA (W3110/pTrcMutmelA), it was determined that it could employ catechol supplemented to the culture medium as a substrate to generate a dark pigment. This product showed similar characteristics to melanin derived from l-tyrosine, such as low solubility in water and a broad band UV–visible absorption spectrum (data not shown). To determine the capacity of strain E. coli W3110/pTrcMutmelA for generating melanin from catechol, resting cell cultures were implemented. Conditions for obtaining a resting cell culture are usually based on the addition of an antibiotic to arrest cell growth [14]. However, it has been observed that strains expressing MutmelA could not grow on minimal salts medium supplemented with glucose as sole carbon source. When l-tyrosine was supplemented to the medium, the strains displayed growth. These results strongly suggest that MutmelA activity is consuming the endogenously generated l-tyrosine, thus causing a phenotype resembling an auxotrophy [14]. Therefore, under culture conditions that lack l-tyrosine in culture medium, the addition of an antibiotic was not necessary to arrest growth of strain W3110/pTrcMutmelA. The inoculum for these cultures was prepared in LB medium supplemented with glycerol 40 g/L, where a final biomass concentration of 7.55 g/L was reached after 15 h (Fig. 2). The resting cell cultures were performed in a bioreactor with modified M9 minimal salts medium containing 40 g/L of glycerol and 0.85 g/L of catechol. The initial E. coli W3110/pTrcMutmelA cell mass was 6.44 g\textsubscript{DCW}/L, displaying a small increase during the experiment (52 h). Under these conditions, 0.43 g/L of supplemented catechol were consumed. At the end of the fermentation, cells were removed from culture medium by centrifugation; the
supernatant pH was adjusted to 2.0 and maintained at 4 °C for 16 h as described in the Methods sections. By following this procedure, 0.29 ± 0.06 g/L of catechol melanin were recovered from the culture supernatant. Melanin concentration in culture medium has been estimated by several groups by measuring absorbance at 400 nm [11–14]. In this work, we calculated that 1 OD at 400 nm measured at the end of the fermentation, corresponds to 0.12 g/L of melanin recuperated after the precipitation procedure. Figure 2 shows the catechol melanin production profile calculated from absorbance art 400 nm. It coincides with catechol consumption from the start of the resting-cell culture until 15 h. The maximum theoretical yield of melanin from catechol is 1 g/g, therefore, the observed yield corresponded to 67 % of the theoretical maximum.

These results indicate that catechol melanin can be produced in a bioconversion process with E. coli W3110/pTrcMutmelA and also suggest strategies for its improvement. Process productivity can be increased by employing higher DCW and catechol concentrations. It has been determined that catechol at a concentration greater than 2.75 g/L has an adverse effect on strains that produce the aromatic intermediate dehydroshikimic acid [24]. By employing catechol feeding strategies, it should be possible to maintain a catechol concentration below the toxic level during the bioconversion cultures.

**Generation of an E. coli strain for the production of catechol melanin from glycerol**

Strains with the capacity of producing catechol from simple carbon sources have been reported [17, 24]. This opens the possibility of developing a process for producing catechol melanin from a renewable raw material. Strain E. coli W3110 trpD9923 is a mutant that overproduces anthranilate [19]. This strain was engineered to overproduce this compound by the overexpression of E. coli genes aroG brk and tktA, encoding a feedback inhibition resistant version of 3-deoxy-d-arabino-heptulosonate 7-phosphate (DAHP) synthase and transketolase, respectively [25]. Expression of an inhibition-insensitive mutant version of DAHP synthase alleviates feedback inhibition, causing an increase in carbon flow from central metabolism to the common aromatic pathway. The increased level of transketolase caused by plasmid expression of gene tktA provides erythrose 4-phosphate (E4P), a direct precursor of DAHP (Fig. 1) [20, 26]. Further modification of this strain by overexpression of genes antABC encoding the components of anthranilate 1,2-dioxynase from Pseudomonas aeruginosa PAO1 resulted in an E. coli production strain with the capacity to synthesize catechol from glucose [17]. To generate an E. coli strain capable of transforming a simple carbon source to catechol melanin, the gene MutmelA was integrated into the chromosomal lacZ gene of W3110 trpD9923, resulting in W3110 trpD9923 MutmelA. This strain was transformed with plasmids pJLBaroG brk tktA and pTrc-ant3 to cause an increase in carbon flow from central metabolism to the synthesis of catechol (Fig. 1).

**Bioreactor cultures for catechol melanin production from glycerol with strain W3110 trpD9923 MutmelA/pJLBaroG brk tktA/pTrc-ant3**

To determine the capacity of strain W3110 trpD9923 MutmelA/pJLBaroG brk tktA/pTrc-ant3 for synthesizing catechol melanin, bioreactor cultures were performed with glycerol as carbon source. Glycerol was chosen since it is an abundant and low-cost raw material that is derived from biodiesel production [27]. An advantage of using glycerol as carbon source when compared to glucose is based on its mechanism of internalization. In E. coli, the phosphoenolpyruvate-sugar phosphotransferase system consumes a mol of aromatics precursor PEP for each mol of imported glucose [20]. As consequence, this transport mechanism limits the yield of aromatics production. In contrast, glycerol internalization does not consume PEP, so a higher aromatics yield can be expected when compared to the use of glucose as raw material. In
these cultures, yeast extract was also employed, since strain W3110 trpD9923 is an L-tryptophan auxotroph. Batch cultures were performed with strain W3110 trpD9923 MutmelA/pJLBaroGₚr₂tkA/pTrc-ant3 in modified M9 medium supplemented with 40 g/L glycerol and 2 g/L yeast extract. Under these conditions, a growth phase was observed until approximately the 17th h, when the culture entered the stationary phase until the end of the culture at 72 h (Fig. 3). In these experiments, 35 g/L of glycerol were consumed. The accumulation of catechol was observed starting at 12 h until the end of the culture, where the titer was 0.73 g/L. The accumulation of melanin was observed, as evidenced by a change to a dark colour of the culture medium starting around the 18th h, closely coinciding with the start of the stationary phase (data not shown). The amount of melanin recuperated at the end of the culture after precipitation from the total culture supernatant corresponded to a concentration of 1.21 ± 0.24 g/L. The catechol melanin production profile was calculated from the absorbance at 400 nm of culture samples (Fig. 3). It can be observed that catechol melanin concentration at the end of the culture corresponded to 0.5 g/L. This value is lower than the 1.21 g/L recuperated after precipitation of the culture supernatant. This discrepancy can be explained considering that melanin formation depends on the non-enzymatic oxidation of precursors generated by the tyrosinase activity. Some of these precursors are likely not detected at an OD of 400 nm. However, when the culture supernatant is subject to a process that involves a total of 32 h of acid precipitation, this allows for a more complete oxidation of melanin precursors. Therefore, it can be assumed that a significant amount of the melanin recuperated after the pH adjustment process is not detected at 400 nm. These results indicate that gravimetric determination of melanin should be favoured over its quantification by absorbance. It should be noted that the process of melanin formation by microbial cultures is still very poorly understood. It remains to be determined how melanin precursors exit the cell as well as the kinetics of the steps leading to the generation of the aromatic polymer. This process is further complicated by the simultaneous existence of diverse molecular weight forms of melanin, each with distinct physical and chemical characteristics.

It is not possible to provide an accurate estimation of the catechol melanin yield from glycerol since this substrate is being consumed for both cell maintenance during the stationary phase and catechol synthesis. Yeast extract (2 g/L) was added to the culture medium in these experiments since strain W3110 trpD9923 MutmelA is a L-tryptophan auxotroph. To determine if yeast extract was contributing with precursors for melanin formation, a control culture was performed with strain W3110/pTrcMutmelA under the same conditions employed for melanin production from glycerol. It was determined that 0.06 ± 0.01 g/L of melanin were produced under these conditions. These results indicate that approximately 95% of the generated melanin is derived from catechol in the cultures with strain W3110 trpD9923 MutmelA/pJLBaroGₚr₂tkA/pTrc-ant3.

The accumulation of catechol in these cultures indicates that its rate of production surpasses the rate of consumption by tyrosinase (Fig. 3). These data clearly point to tyrosinase activity as an important target for strain optimization. Potential improvement of the production strain could be achieved by exploring gene expression and mRNA optimizations strategies based on synthetic biology approaches. Also, a gene encoding one of the tyrosinases from organisms that naturally produce melanin derived from catechol could be expressed and evaluated in these E. coli strains with the aim of improving catechol melanin productivity and titer [15, 16].

Characterization of the produced melanin
IR spectra were determined from melanin samples produced from (1) L-tyrosine (eumelanin) by previously reported recombinant E. coli strain [14], (2) glycerol-derived catechol by strain W3110 trpD9923 MutmelA/pJLBaroGₚr₂tkA/pTrc-ant3 and (3) supplemented catechol
by strain W3110/pTrcMutmelA (Fig. 4). A broad band at 3200–3400 cm\(^{-1}\) is evident for all samples, although for catechol melanins generated by bioconversion or total synthesis from glycerol, a band at 3274 cm\(^{-1}\) is more visible. It relates to stretching vibrations from—OH and –NH groups. Other evident differences can be noticed from the melanin samples produced from catechol or glycerol, compared against the l-tyrosine-derived melanin spectrum, such as the presence of two bands located at 2924 and 2853 cm\(^{-1}\), assigned to the stretching vibration of aliphatic –CH, along with a 1454 cm\(^{-1}\) band attributed to C–H bending. Chen et al. suggest that these bands, absent in spectrum a, are related to a characteristic difference between l-tyrosine or L-DOPA, and catechol melanins, which are known to be present in *Rhizoctonia* and *Ustilago* species [15]. Also noticeable for samples b and c are the intense bands at 1644 cm\(^{-1}\) due to aromatic C=C and –COO– vibrations and the peaks at 1515 cm\(^{-1}\) and 1216 cm\(^{-1}\), corresponding to C=O stretching and OH deformation in carboxyl groups. All of these are absent in l-tyrosine or L-DOPA-derived melanin. These results show that the two melanin samples, generated from supplemented catechol or from catechol synthesized in vivo by the metabolically engineered production strain, show the main expected absorbance peaks of functional groups for a catechol polymer.

**Conclusions**

Melanins are natural polymers having a broad range of potential applications. However, their widespread utilization is limited by the availability of a well-characterized product obtained at a relatively low cost. This is not the case for eumelanin since several microbial strains and biotechnological processes for its industrial production have been reported [11–14]. In contrast, the number of production strains and processes for other types of melanins is very limited. There is only one report on the production in a liquid medium of a melanin derived from homogentisic acid (pyomelanin). By employing the mutant *Pseudomonas putida* strain F6-HDO, 0.35 g/L of pyomelanin was produced [30]. In the case of allomelanins, this report constitutes the first example towards employing a microbial strain developed by metabolic engineering for the gram-level production of catechol melanin from glycerol. These efforts will contribute to a better understanding of the distinct properties of various melanins, leading to potentially unique industrial applications.

**Abbreviations**

- Ap: ampicillin; Tc: tetracycline; Gm: gentamicin sulphate; LB: Luria–Bertani; 
- OD\(_{600}\): optical density at 600 nm; 
- HPLC: high performance liquid chromatography; 
- DCW: dry cell weight; 
- FTIR: Fourier transform infrared spectroscopy; 
- GlpD: glycerol transporter; Gly: glycerol; Gly3P: glyceraldehyde-3-phosphate; 
- G6P: glucose-6-phosphate; 
- PPP: pentose phosphate pathway; 
- E4P: \(\delta\)-erythrose 4-phosphate; 
- PEP: phosphoenolpyruvate; 
- DAHP: 3-deoxy-o-arabino-heptulosonate 7-phosphate; 
- CHA: chorismate; 
- l-Trp: l-tryptophan.

**Authors’ contributions**

AMC, RDA, VMC and GG participated in the design of this study. AMC constructed the strains. AMC, RDA, GHC and CAV characterized the strains in flask and bioreactor cultures and analyzed the experimental data. SR and GHC participated in performing FTIR analysis of catechol melanin samples. AMC, RDA, SR, GHC, CAV, AM, FB, VMC and GG participated in the analysis of the results as well as in writing and critical review of the manuscript. All authors read and approved the final manuscript.

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