



Antioxidant Activity and Role of Culture Condition in the Optimization of Red Pigment Production by *Talaromyces purpureogenus* KKP Through Response Surface Methodology

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Abstract

The red pigment production by *Talaromyces purpureogenus* KKP, a soil isolate, was optimized by response surface methodology (RSM) in the present study. The cultural parameters, such as pH, temperature, dextrose, and peptone concentrations, were optimized for red pigment production using the central composite design (CCD) experimental design. A second-order quadratic model was used to calculate the relationships between the values at different levels of response. The optimum values of the selected variables under coded factors are 6.0, 27 °C, 2.25%, and 1.10% for pH, temperature, dextrose, and peptone, respectively. The selected variables were most effective in the enhancement of red pigment production at optimized culture conditions. In addition to optimization, the antioxidant activity of the pigment isolated in the present study was found to be promising with IC₅₀ value (40 µg/ml). The HRMS data revealed the identification of delphinidin, limonene, 6-hydroxymethyl-7,8-dihydropterin, D-mannose 6-phosphate, and CDP-DG (18:0/18:0). The results of the present investigation will be added to the existing literature of red pigment production and its optimization by *T. purpureogenus*

Introduction

Filamentous fungi belonging to the genus *Monascus*, *Aspergillus*, *Fusarium*, *Penicillium*, *Trichoderma*, *Laetiporus* are well known for the production of colored pigments [1, 2]. The specific pigment molecules and groups like polyketides and anthraquinones can be separated [3–5]. These pigments are finding application in food, cosmetics, pharmaceuticals, and textile industries. The adverse effects of synthetic colorants can overcome by using the natural pigments of fungal origin. In addition to their coloring capacity, it also poses several bioactivities [6].

Among the aforementioned fungal genus, several species of *Monascus* produce more than 90 distinct pigments [7–9]. The six major pigments of *Monascus* sp. and its color are ankaflavin, monascine (yellow), rubropunctatin, monascorubrine (orange), rubropuntantamine, and monascorubramine (red) [10]. However, *Monascus* sp., also produces mycotoxin called citrinin in addition to the drug mevinolin during pigment production [11, 12]. Citrinin is a yellow pigment and a known carcinogen with nephrotoxic, hepatotoxic, and cytotoxic activity [13]. These unwanted metabolites restrict its usage in the commercial production of pigment for various applications. At the same time, the production of monascus like pigments by *Penicillium* (and or *Talaromyces*) species is well reported in the literature [14, 15].

Several species of *Penicillium/Talaromyces* are reported to produce red pigments [14, 15]. For the industrial production of pigments, it is important to have a superior isolate and an economically viable process (media and cultivation conditions). The production of pigment is influenced by several factors [1, 16–19], including the inherent quality of the isolate. Fungi are one of the most biodiverse organisms on earth, with an estimate of 2.2 to 3.8 million species [20]. The huge diversity of fungi with numerous species belonging to particular genera with diverse metabolism produces an array of secondary metabolites [21]. Consequently, there is every

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possibility of finding an isolate with superior performance than the existing one, which is worth investigation. In this milieu, the search for an ideal, industrially relevant isolate producing the red pigment continues. Above all, the whole process of can be optimized using statistical tools like RSM [22]. Previously, the usefulness of RSM in optimizing the pigment production by *Penicillium* isolates has been already demonstrated [23–25], but the cultural parameters and substrates were different. In the present study, the process of red pigment production by *T. purpureogenus* KKP was optimized by RSM and also its antioxidant activity was reported.

Methods

Isolation and Maintenance of Pure Culture

Talaromyces purpureogenus KKP (Fig. 1) was isolated from the soil obtained from Keekan village (12°22'23.43" N 75°03'38.89" E), Kasaragod district, Kerala, India. The isolation was carried out by serial dilution and pour plate method using Potato Dextrose Agar (PDA) (Hi media, Bombay, India) as isolation media. The pure culture of *T. purpureogenus* was maintained on PDA agar slants at 4 °C for further studies and laboratory code was given as KKP.

Characterization (Macroscopic) of *T. purpureogenus* KKP

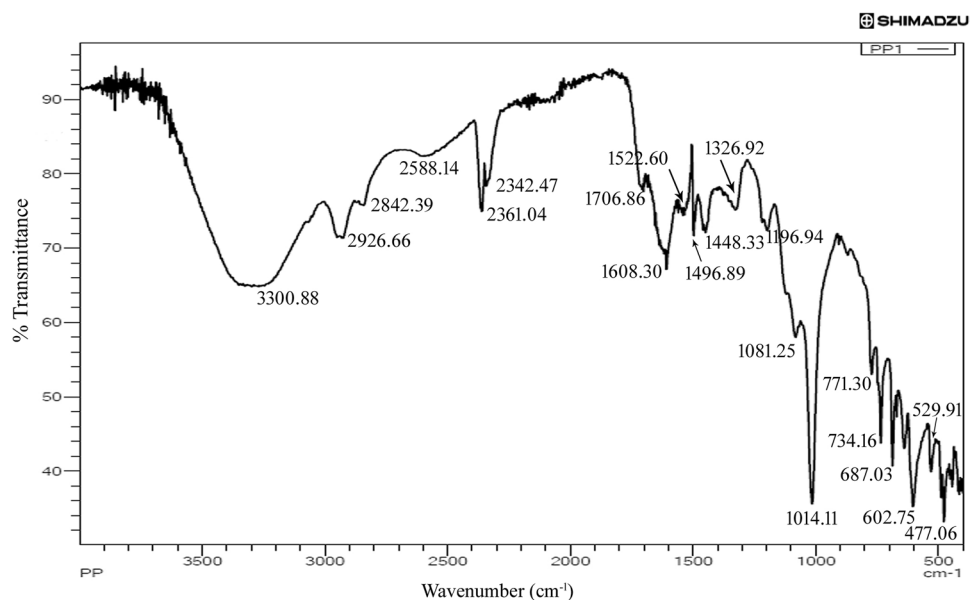
Macroscopic (colony character) features (color, texture, and topography) of the colony were studied using four standard media namely Potato dextrose agar (PDA), Sabouraud's dextrose agar (SDA), Czapek's dox agar (CDA), and V-8 juice

agar (V8JA). The spore suspension was prepared from the seven days (28 ± 1 °C) old *T. purpureogenus* KKP culture on PDA plate using the solution containing 0.05% Tween 80 by gently swirling the plate. The hyphal fragments and other debris were removed by filtering the spore suspension through sterile absorbent cotton wool using a funnel. The concentration of spores was adjusted to 1×10^6 spores/ml using hemocytometer and light microscope. Ten microliters of the suspension was spot inoculated on the selected media and incubated at 28 °C for 7 days.

Molecular Identification of *T. purpureogenus* KKP

Talaromyces purpureogenus KKP DNA was extracted using a DNA extraction kit (HiPurA™ Himedia, India) according to the manufacturer's instructions. The internal transcribed spacer (*ITS*) region 1 and 4 of DNA was utilized for identification [26]. The sequence of universal primers used for the PCR amplification was ITS1—5'-TCCGTAGGT GAACCTGCGG-3' and ITS4—5'-TCCTCCGCTTATTGATATGC-3'. The PCR was carried out containing 2 × PCR master mix (Genei, Bangalore, India), forward and reverse primers (10 pmol/μl), template DNA (12–15 ng/μl) at 95 °C for 10 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min and finally at 72 °C for 8 min for extension. The PCR products were sequenced, and the obtained sequence was analyzed using BLASTN (<https://www.ncbi.nlm.nih.gov/blast/Blast.cgi>). Soil fungi were identified based on the percentage of homology to sequences available in the database, and CLUSTAL W was used for alignment of closely related soil fungal sequences, and the sequence was submitted to the GenBank database. Sequences with maximum identity to that of the *T.*

Fig. 1 FTIR spectrum of the red pigments produced by *T. purpureogenus* KKP



purpureogenus were retrieved from the GenBank. Twenty of the closest sequences within the same taxon and related taxa were selected for the evolutionary analysis, and the tree was generated using MEGA 7 software [27].

Red Pigment Production

For red pigment production, 500 μl of spores (1×10^6 spores/ml) of *T. purpureogenus* KKP was inoculated into 250-ml Erlenmeyer flasks containing 100 ml of sterile (121 °C for 20 min) minimal medium containing (per litre), NaCl 1 g; KCl 0.5 g; MgCl_2 0.4 g; CaCl_2 0.1 g; Na_2SO_4 0.15 g; KH_2PO_4 2 g; Na_2HPO_4 2.25 g and trace elements 1 mg FeCl_3 , 0.1 g MnCl_2 and 46 mg ZnCl_2 per litre [19]. The pH of the media was adjusted with 0.1 mol/L HCl or NaOH, before sterilization. The inoculated flasks were incubated with shaking (120 rpm) at 30 °C for 72 h.

Extraction of Red Pigment

After the incubation period, the whole culture was filtered through Whatman No one filter paper. The filtrate was centrifuged at 10,000 rpm for 15 min. The supernatant was saved and the pigments were concentrated by Speed Vac Concentrator (Thermo Fisher Scientific, USA).

Characterization of Red Pigment

Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

A known concentration (1 mg/ml) of the pigment was suspended in methanol and analyzed for functional groups by FTIR-Spectrophotometer (Shimadzu, Japan) in the spectral range 450 to 4000 cm^{-1} . The obtained spectrum was analyzed for the presence of possible functional group and compared with the red pigments produced by other fungal isolates, including *Talaromyces* sp.

High-Resolution Mass Spectrometry (HRMS) Analysis

The partially purified pigment extract was dissolved in 1.0 ml of MS grade 0.1% formic acid (1 mg/ml). The sample was filtered by using 0.45 μm syringe filter and further diluted to 1:1500 in MS grade 0.1% formic acid before subjecting to HRMS analysis (ABSIEX QTRAP 6500). 5 μl of sample was injected into the HRMS system, fitted with an electron spray ionization source. The flow rate was maintained at 5 $\mu\text{l}/\text{min}$. The data were acquired in positive mode by using the Analyst software (Analyst Version 1.6.2). The spectrum was recorded against intensity, versus m/z , Da. The mass of the sample was set in the range of 100–1000. The MS parameters set as declustering potential (DP) of 100 V, collision energy (CE) of 40 V, and ion spray voltage was set

at 5500 V. Yeast metabolome database was used for metabolite identification (YMDB version 2.0).

Estimation of Red Pigment

The production of red pigments in the culture media was estimated by spectrophotometric measurements (from 350 to 500 nm). The amount of red pigment in the culture media (concentrated through Speed Vac Concentrator and diluted 1:10) was determined by taking the absorbance at 500 nm and expressed in terms of color value unit /mL according to a method previously reported (with some modifications) [28]. The color value was calculated as stated below,

Color value unit/ml = Absorbance at 500 nm \times dilution factor \times 1000/sample volume (μl).

Experimental Design for the Selection of Parameters

The two cultural conditions and two media components were selected for the optimization process based on relative pigment production estimation. The selected fungal strain known to utilize the two media component as major determinant for pigment yield dextrose (0.5 to 4%) and peptone (0.2 to 2%), and culture conditions like pH (4 to 8) and temperature (22 °C to 32 °C) were included for the analysis of red pigment production by RSM method.

Response Surface Methodology (RSM)

The central composite design (CCD) was applied to optimize four different selected parameters for the optimization of cultural conditions of red pigment production. The responses by red pigment production on selected parameters were designed by RSM. Thirty experiments with six replications at the center point were designed for pH (X_1), temperature (X_2), dextrose (X_3), and peptone (X_4) at five coded levels ($-\alpha$, -1 , 0 , $+1$ and $+\alpha$). All the experiments for the relative production (U/ml) of red pigment were performed in duplicates, and average values are presented in Table S2. For the RSM statistical calculation, the experimental variables coded x_i for X_i as according to the following equation:

$$x_i = (X_i - X_0) / \Delta X \quad (1)$$

where x_i is considered for the coded value of the variable X_i , X_0 is considered for the value of X_i at the center point, and ΔX is considered for the step change value.

The experimental data were further analyzed using multiple regression analysis, and second-order polynomial model was fitted to response giving the following equation.

$$Y = \beta_0 + \beta_i X_i + \beta_{ii} X_i^2 + \beta_{ij} X_i X_j \quad (2)$$

where Y is the predicted response of produced red pigment, β_0 is the intercept coefficient, β_i is the linear coefficient, and β_{ii} is the quadratic coefficient, and β_{ij} is the interaction coefficient.

Determination of DPPH and ABTS Scavenging Activity of Red Pigment

The red pigment was evaluated for its ability to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma) radicals as per the previous method [29]. In brief, different concentration of red pigment (0.6 to 100 $\mu\text{g/ml}$) was added into a 0.2 mM DPPH solution and incubated at 28 °C for 30 min. The pigment absorbance (A_S) and blank absorbance (A_B) were measured at 517 nm. The DPPH scavenging capacity (SA_{DPPH}) was calculated using the following formula:

$$SA_{\text{DPPH}} = A_B - A_S / A_B \times 100$$

Similarly, 2,2'-azinobis 3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay was performed as per previously reported method [30]. The ABTS radical cation was produced with 2.45 mM potassium persulfate in the dark room for 16 h. The red pigment was added to ABTS solution at different concentration (0.6 to 100 $\mu\text{g/ml}$) and incubated at 28 °C for 5 min. The absorbance of the reaction mixture was measured at 734 nm and compared with the standard (Ascorbic acid).

Statistical Analysis

The data from the central composite design, as shown in Table S2, were used to determine the regression coefficients of the second-order multiple regression analysis. The goodness-of-fit of the regression model and the significance of the parameter estimates were determined by the analysis of variance (ANOVA, Table S3). The Design-Expert 7.0 software was employed for statistical analysis.

Results

Isolation and Morphological Characterization

Talaromyces purpureogenus was originally isolated from clay soil and was characterized based on the colony appearance (Fig. S2) and by sequencing the ITS region of the DNA. *T. purpureogenus* grew rapidly on SDA and PDA when compared to the other two (V8JA and CDA) media. The average size of the colonies on PDA and SDA was 45 and 51 mm in diameter, respectively, when incubated at room temperature (28 °C for 7 days). The colony was radially sulcate (the grooves were prominent and well pronounced on SDA),

velutinous, predominantly greyish turquoise/green (in the periphery) with a white margin. On the reverse side, the colony appeared mahogany/reddish-brown in color. Diffusion of red pigments in the media can be seen. The concentration of red pigment was higher in SDA when compared to PDA. The growth of *T. purpureogenus* KKP (31 mm) and CDA (39 mm) was moderate. The color of the colony on V8JA was greyish turquoise without a white margin with diffused red pigments around the colony. The colony of *T. purpureogenus* KKP appeared distinctly on CDA, with pale yellowish-cream in color (with scanty red pigments around the colony), probably due to the lack of pigmentation. The reverse side of both V8JA and CDA appeared dark red in color.

Identification of Fungal Isolate

The closely related soil fungi from BLASTN database were used to construct Phylogeny using the maximum likelihood method based on the Kimura 2-parameter model tested with 1000 boot strappings. Phylogenetic analysis (Fig. S3) based on the ITS sequence of our isolate with the closest BLAST hits showed that isolate KKP was more homologous to a *T. purpureogenus* (GenBank accession no. EU427301.1 and AY373926.1) sequences available in the database.

Pigment Production

In order to understand the pigment production by *T. purpureogenus* KKP under normal conditions, a preliminary study was carried out using potato dextrose broth at 28 °C, incubated for 72 h with shaking at 120 rpm. The unit value produced was very low (3.6 U/ml), which was not suitable for industrial purposes. Therefore, the media with abundant dextrose and nitrogen content along with pH and temperature was utilized for the optimization of red pigment production.

FTIR Analysis

FTIR analysis of the pigment showed a strong, broad peak at 3300 indicating the O–H stretch and the wavenumbers 2926 and 2842 indicate CH stretch (due to $-\text{CH}_2$ group) (Fig. 1). The absorption peaks between 1608 and 1448 correspond to the aromatic ring C=C vibration bands and 1196 relate to C–O stretching vibration. The peak at 1448 may also be due to $-\text{C}-\text{H}$ bending (in CH_3 groups)/stretching vibration of aliphatic side chains. The medium peak at 1522 might represent C=C stretching vibration of aliphatic side chains. Strong peaks at 1081 and 1014 indicate C–O–C stretch/C–O group.

HRMS Analysis

Our HRMS analysis revealed the identification of several metabolites from the partially purified pigment extract. We have identified 141 metabolites from the pigment extract at MS1 level. We have identified delphinidin with precursor 189 m/z, is a anthocyanidin belongs to polyphenol family. We are also identified limonene with precursor mass 432.2 m/z, which belongs to class of organic compounds known as 3-alkylindoles. We have identified 6-hydroxymethyl-7,8-dihydropterin with precursor mass 432.2 m/z, which belongs to class of organic compounds known as pterins and derivatives. It mainly involved in the metabolic pathway called tetrahydrofolate biosynthesis pathway in many fungus species including yeast. Apart from this we also identified D-mannose 6-phosphate and CDP-DG (18:0/18:0) with precursor mass 305 and 526.3 m/z, respectively. D-mannose 6-phosphate belongs to the class of hexose phosphates and mainly involved in mannose metabolism pathway (Fig. S1). The complete lists of identified metabolites are given in table (Table S1).

Optimization of Pigment Production: Central Composite Design

As described above, the model was designed with CCD to observe the relative importance of cultural conditions to produce red pigment. The red pigment production was measured with respect to difference between variables at high level and low-level. The red pigment production had a wide variation from 0.6 to 138.3 U/ml (Table S2). This production variation suggests that the importance of cultural condition optimization for the higher production of red pigment from the strain *T. purpureogenus* KKP. The observed model showed the significance based on the calculated p value, in the variation of cultural conditions such as temperature, pH, dextrose, and peptone concentration (Table S3).

Response Surface Methodology

Red pigment has many biotechnological applications with the media formulation and composition may confer stability, yield of production and mass production. Based on CCD model, pigment production of 138.3 U/ml was optimized at the cultural condition of $X_1 = 6.0$, $X_2 = 27^\circ\text{C}$, $X_3 = 2.25\%$, and $X_4 = 1.10\%$. The results were fitted with following second-order polynomial equation based on regression coefficient, the response variable and the test variables:

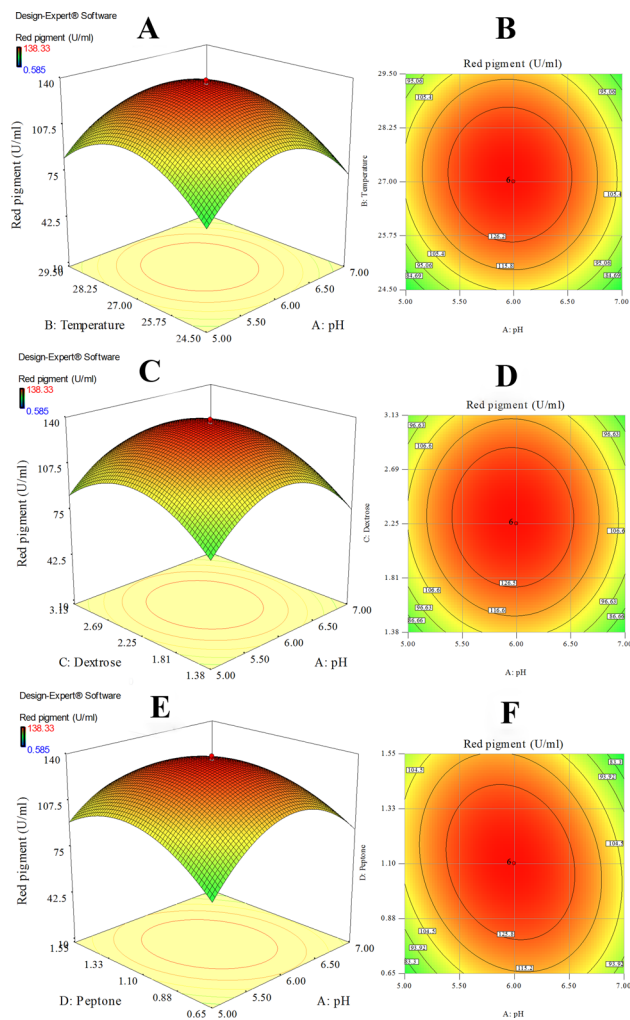


Fig. 2 Response surface plots (a, c and e) and 2-D contour plots (b, d and f) of red pigment produced by *T. purpureogenus*. The plots show the interaction between temperature and pH (a and b), dextrose and pH (c and d), peptone and pH (e and f) (Color figure online)

$$\begin{aligned}
 Y = & (-395.09546 + 48.36836)X_1 + 16.56266X_2 + 18.94804X_3 \\
 & + 35.13961X_4 - 0.25480X_1X_2 - 0.62707X_1X_3 - 1.76194X_1X_4 \\
 & + 0.0099X_2X_3 + 0.31422X_2X_4 - 0.32365X_3X_4 - 3.22098X_1^2 \\
 & - 0.28379X_2^2 - 3.42528X_3^2 - 14.39774X_4^2
 \end{aligned} \quad (3)$$

The statistical significance of quadratic model was checked by F test and p value, and the ANOVA to understand adequate prediction through the response surface method (Table S3). The model was adequate for the prediction of experimental variables as indicated by regression

coefficient R^2 was 0.9113. ANOVA of the quadratic regression model demonstrated that the model generated is highly significant, as indicated by F test with a low probability value ($p_{\text{model}} < 0.0001$). The linear coefficients (X_1, X_2, X_3, X_4), a quadratic term coefficient ($X_1^2, X_2^2, X_3^2, X_4^2$), and the interaction coefficient (X_1X_3) were found to be significant ($p < 0.0001$). Based on Eq. (3) the full model was made three-dimensional and contour plots to predict the relationship between independent and dependent variables (Figs. 2 and 3).

Optimization of Cultural Conditions

The result of predicted model and red pigment production based on cultural condition optimization by RSM is shown in Figs. 2 and 3. The plots show the effects of selected two factors on the response at a time in 3-D response surface

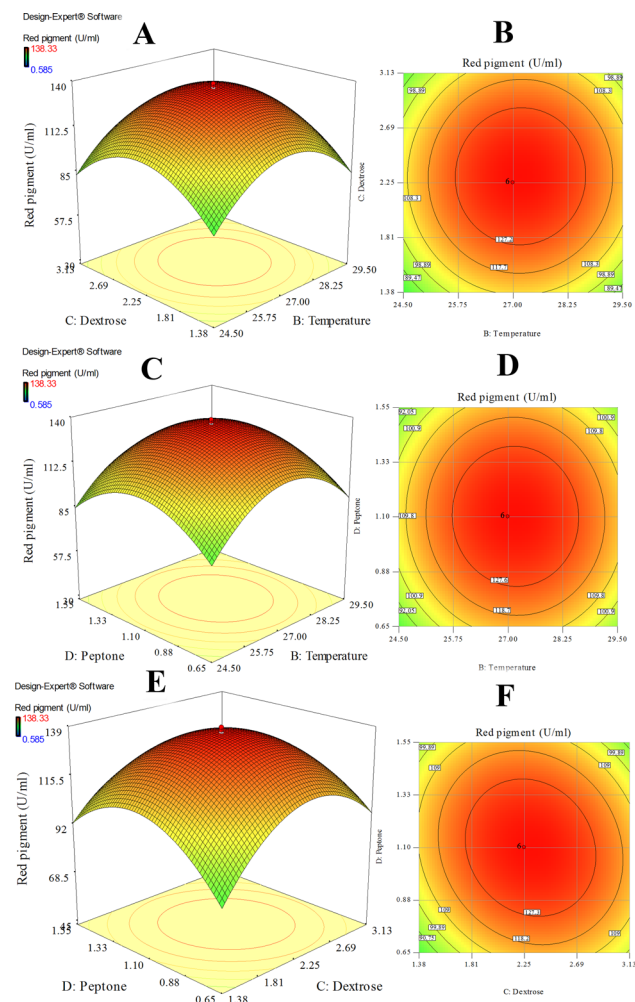


Fig. 3 Response surface plots (a, c and e) and 2-D contour plots (b, d and f) of red pigment. The plots show the interaction between dextrose concentration and temperature (a and b), peptone and temperature (c and d), peptone and dextrose (e and f) (Color figure online)

and 2-D contour plots. The contour plots were observed to analyze the interaction of the variables and to predict their optimum levels for maximum production of red pigment. Figure 2 shows the effect of pH and temperature on the red pigment production along with dextrose and peptone concentration. These plots indicate that the production of red pigment is highly influenced by temperature and pH as well as dextrose and peptone concentration. The red pigment production was increased with increase in the pH of media up to the optimum level of pH 6.0, and a further increase in pH resulted in a decrease in pigment production. Similarly, an increase in temperature resulted in an increase in pigment production up to the optimum level of 27 °C, and a further increase in temperature suggested decrease in total pigment units/ml.

Similarly, dextrose and peptone concentration in the media were optimized as 2.25% and 1.10%, respectively. Figure 3 shows the interaction between temperatures, dextrose, and peptone concentration in 3-D response and 2-D contour plots, respectively. The dextrose and peptone concentration are also influenced by changes in pH as well as incubation temperature. Therefore, the optimum cultural conditions proved to be pH, temperature, dextrose, and peptone concentration were 6.0, 27 °C, 2.25%, and 1.10% respectively. The RSM was employed to optimize the cultural conditions for red pigment production by *T. purpureogenus* KKP. The RSM model showed the value of R^2 0.9113 and the optimized cultural conditions, dextrose, and peptone supplementation exhibited 38-fold increases in red pigment production (Fig. S4). To confirm the fold change in the red pigment production, the selected parameters were repeated and red pigment production was measured with respect to change in pH, temperature, glucose, and peptone concentration (Fig. 4).

Antioxidant Activity of Red Pigment

Radical scavenging capacity of the red pigments was measured through DPPH assay at different concentrations with ascorbic acid as a standard. As indicated in Fig. 5, the DPPH scavenging rate of the red pigment ranged from 10 to 90% with IC_{50} (half inhibition concentration) value of 40 $\mu\text{g/ml}$ and the standard ascorbic acid was shown as IC_{50} value 7.25 $\mu\text{g/ml}$ (Fig. 5a). In ABTS assay, IC_{50} of ascorbic acid was 7 $\mu\text{g/ml}$, while that of the red pigment was 35 $\mu\text{g/ml}$ (Fig. 5b).

Discussion

Filamentous fungi produce a broad range of pigments (monascins, carotenoids, melanins, flavins, phenazines, quinones, violacein, or indigo). Several factors play an

Fig. 4 Effect of temperature, pH, carbon (dextrose), and peptone supplementation on the red pigment production (Color figure online)

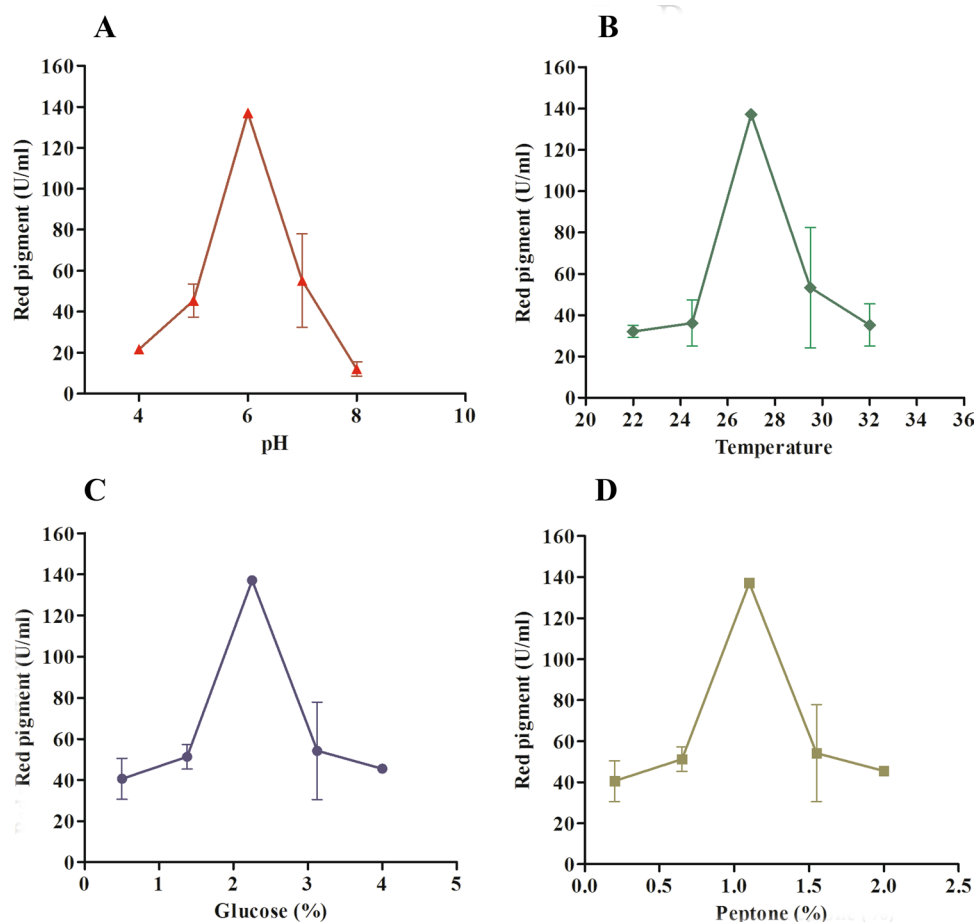
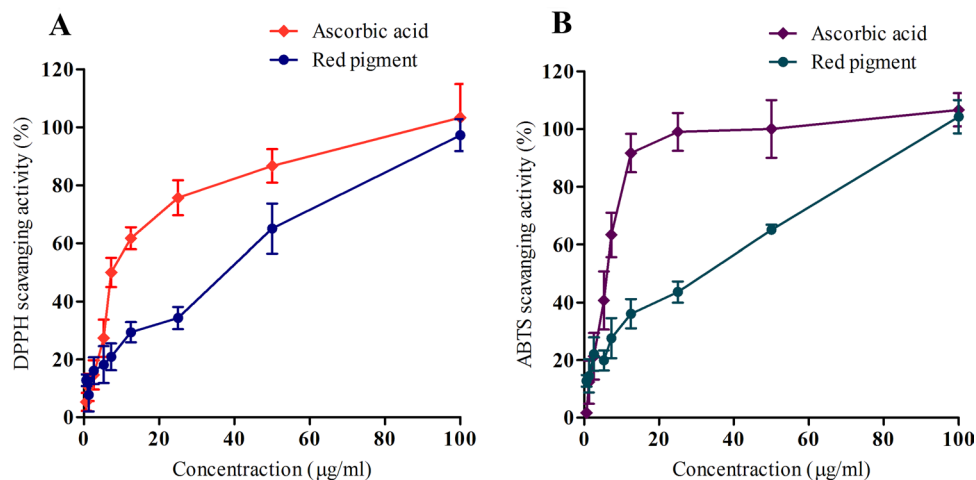


Fig. 5 Antioxidant activity of red pigment from *T. purpurogenus* KKP measured by DPPH (a) and ABTS (b) scavenging assay (Color figure online)



important role in the economical production of microbial pigment. A superior microbial isolate producing the pigment, an optimized media, and the method of downstream processing (purification) is an important one. Among the factors mentioned above, a competent microbial isolate

is one of the most important one. A superior isolate with rapid growth, tolerance to high concentrations of carbon or nitrogen, having the ability to metabolize inexpensive substrate, without undesirable (toxic) products are the desirable traits in an industrial bioprocess [31].

Strain improvement is one of the strategies to improve the yield of the product. However, the disadvantages of strain improvement such as introduction of the unwanted mutations limit its utilization. Exploring the huge fungal biodiversity may yield strains with desirable qualities because the variation in wild populations is expected due to difference in the genetic/phenotypic variations, which in turn may give rise to a competent strain with desirable qualities [36]. Therefore, in the present study, *T. purpurogenus* KKP, a wild isolate was studied for its pigments production and antioxidant activity.

Phylogenetic analysis showed *T. purpurogenus* KKP closest relationship with *P. purpurogenum* FRR 1147. The new classification of Samson et al. (2011) [32] recombines the *Penicillium* subgenus *Biverticillium* species into *Talaromyces* and in the new scheme, *P. purpurogenum* is known as *T. Purpurogenus*. According to the report of Yilmaz et al. (2014) [33] based on the Internal Transcribed Spacer (ITS) region, β -tubulin (BenA) and DNA-dependent RNA polymerase II second largest subunit (RPB2) gene sequences of 88 accepted *Talaromyces* species, *T. purpurogenus* finds place in the clade *Talaromyces*. Others are *Helici*, *Purpurei*, *Trachyspermi*, *Bacillispori*, *Subinflati*, and *Islandici*. Further, *P. crateriforme*, and *P. sanguineum* shares close relationship with *T. purpurogenus* (Yilmaz et al. 2012), which is based on the polyphasic classification which includes macro- and micro-morphology, extrolite production, and multigenes (β -tubulin, calmodulin, DNA-directed RNA polymerase II largest (RPB1), and second largest subunit (RPB2)).

FTIR analysis confirmed the presence of red pigments produced by *T. purpurogenus* KKP. The spectra were matching with the previous report spectra [24], whose works dealt with the red pigment production of *Penicillium* sp. (GBPI_P155) and *Monascus ruber* Tieghem IOC 2225 respectively. Most of the peaks were matching with the earlier works except two small, but sharp doublets at 2361 and 2342, possibly an interference due to carbon dioxide (CO₂) present in the sample atmosphere which gives rise to absorption in IR spectra. Peaks between 1496 and 1608 correspond to aromatic ring C–C vibration, which are characteristic for the aromatic benzene ring. Several peaks were observed in the fingerprint region (1450 to 500) [34]. The spectra in this region are due to the bending vibrations (out-of-plane deformation) within the molecule. The peaks in the 1430–1470 regions were due to the symmetrical deformation of –CH₂ group. Sharp peaks at 1014 and at 1081 were also observed, probably due to C–C–O stretch/bends. Overall, the above spectral information confirms the presence of quinone and phenolic structures, part of the bicyclic azaphilone skeleton of monascus azaphilone pigments [35]. This data was well supported by identification of delphinidin, limonene, and 6-hydroxymethyl-7,8-dihydropterin through HRMS analysis.

The red pigment has many biotechnological applications. Statistical optimization of cultural conditions is one of the valuable tools for getting maximum production of pigments from a microbial source. Most of the optimization of media component and conditions in the recent reports relied on statistical experimental design and response surface analysis. CCD was applied for screening of culture condition and supplements for the media and its analysis showed significance of selected variables. The designed variables subjected to statistical and mathematical optimization resulted in optimized condition for the production of red pigment from the *T. purpurogenus* KKP. Moreover, this empirical technique of optimization enables to evaluate the significance of designed model and interaction between the independent variables (Figs. 2 and 3). The interaction plots suggest that the selected variables like temperature, pH, dextrose and peptone significantly affect the yield of the red pigment production (Fig. 4). Interestingly, this model showed the value of R^2 as 0.9113 and the optimized cultural conditions exhibited 38-fold increases in red pigment production. This fold increase in the total yield is highly recommendable in the utilization of designed method for the red pigment production in industrial scale compare to other reported optimization methods [2, 25]. Furthermore, screening of red pigment for its antioxidant activity suggested that presence of significant lower IC₅₀ value indicating the added advantage in the use of pigment as food colorant [18].

Conclusion

In the current study, red pigment production by *T. purpurogenus* KKP was successfully enhanced by RSM. It was possible to produce and increase the red pigment production by 38 fold. Culture conditions such as pH, temperature, dextrose, and peptone concentration were optimized to 6.0, 27 °C, 2.25%, and 1.10%, respectively, for the enhanced production of red pigment (138.3 U/mL). The present study greatly encourages the transfer of process for red pigment by *T. purpurogenus* KKP for commercial exploitation.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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