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Isolation and identification of *Talaromyces purpurogenus* and preliminary studies on its pigment production potentials in solid state cultures

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Pigments from various sources such as annatto seeds, cochineal, beet root, and microalgae are widely used in food, pharmaceutical, cosmetics, textile and other industries. However, these sources of pigments have various limitations such as toxicity and environmental pollution of synthetic pigments, and low productivity of pigments from higher organisms due to long period of growth. The objective of this study was to screen for pigment producing fungi to overcome some of the above limitations. A pigment producing fungus was isolated from soil sample collected from cassava processing site and was identified as *Talaromyces purpurogenus* based on the colony morphology and characteristics, microscopic observation of the conidia and conidiophores and analysis of the gene sequence of internal transcribed spacer (ITS) region of the rDNA. The nucleotide sequence was deposited in Genbank (DDBJ/ EMBL) and was assigned the accession number LC128689. Pigment production by the isolate in solid state cultures using PDA as substrate in Petri dishes was investigated. The optima culture conditions were pour plating method with agar overlay (4 mm thick) and sealed edges, inoculum spore concentration of 2×10^8 spores/Petri dish and incubation in dark at 30°C. Under these culture conditions, the red, orange and yellow pigments produced were 11.2, 7.3 and 8.21 unit optical densities per gram of wet agar respectively after 96 h of cultivation. The isolate has good potential for production of different shades of pigments for various applications.

Key words: *Talaromyces purpurogenus*, pigments production, solid state cultures, pigment-producing fungi.

INTRODUCTION

Pigments are very useful compounds with versatile applications. They can be applied in many areas of human life because of their health benefits, aesthetic and other beneficial values. Pigments find applications in food and feeds (Gupta et al., 2007; Mapari et al., 2010; Manimala et al., 2014), pharmaceuticals, cosmetics,

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wineries and textile (Sharma et al., 2012), for colouring woods for aesthetic values (Robinson et al., 2012), for dyeing of tanned leather (Velmurugan et al., 2009) and for dying textile materials (Poorniamal et al., 2013)

Although many synthetic colourants are widely used in industries because they are relatively cheap, some of them can have some detrimental effects on human health and some of them are carcinogenic. Thus a lot of attention is now focused on natural pigments because many of them are known to play some beneficial roles in human health. Some of them have antioxidant (Manimala et al., 2014; Cassia et al., 2005), antimicrobial (Vendruscolo et al., 2014) and anticarcinogenic properties (Deshmukh et al., 2009). These biopigments can be obtained from various natural sources such as higher plants like elderberries (*Sambucus nigra*) (Szaloki-Dorko et al., 2015), black grape skin, red beetroots (*Beta vulgaris*), oil palm fruits, seeds from annatto (*Bixa orellana)*, paprika (*Capsicum annum* L.) and tomato (*Lycopersicon esculentum*). Pigments can also be obtained from animals such as insects like kermes (*Kermes vermilio*) and cochineal (*Dactylopius coccus*) (Yilmaz et al., 2014). However, production of pigments from these higher organisms is limited by their low growth rates. Growing of plants to maturity takes at least some months and some acres of lands are required for a large scale production. Pigment production from insects such as *Dactylopius coccus* also takes time and a large number of them are required to extract the pigments for large scale production (Nejad and Nejad, 2013). For example, it was estimated that 155,000 insects are required to produce 1 kg of cochineal dye from *D. coccus* (https://www.dirtdoctor.com/garden/Cochneal_vq1276.htm; Nejad and Nejad, 2013)

Other natural sources of pigments are microorganisms such as fungi, microalgae, bacteria and lichen. Researches on microbial pigments have been expanding since the past few decades because pigments from these lower organisms are more reliable than those from the higher plants and animals (Dufosse et al., 2014; Chen et al., 2015; Abdel Ghany, 2015; Vendruscolo et al., 2015). Due to the high growth rate of microorganisms, their pigment productivities are high, and not affected by time of the year and seasons while their production has little or no negative environmental impact (Duran et al., 2002). Among the microorganisms employed in the production of pigments, filamentous fungi have been reported to produce large quantities and varieties of pigments because of their ability to synthesize various enzymes that enable them utilize various substrates and catalyze the synthesis of various compounds. Among the fungal kingdom, filamentous fungi of the class ascomycetes are the most popular group known to produce soluble pigments (Dufosse et al., 2014). The first fungus documented to be used for the production of food grade pigments was *Monacus* species (Tieghem, 1884) and since then many strains of *Monascus* have been isolated,

identified and used for pigment production. Some examples include *Monascus purpureus* strain FTC 5391 (Musaalbakri 2006), *Monascus ruber* (Buhler et al., 2015)*,* and *Monascus* sp. strain M9 (Wang et al., 2015). In addition to *Monascus* species, several other species of filamentous fungi such as *Penicillium aculeatum* ATCC 10409 (Afshari et al., 2015), *Penicillium* sp. DLR-7 (Chintapenta et al., 2014) and *Paecilomyces* sp (Cho et al., 2002) have been used for pigment production.

Although many strains of fungi have been isolated and investigated for pigment production, most of the isolates have one or more of the following limitations: Unattractive colour of the pigments, low productivity, low stability of the pigment at high temperature, extreme pH etc., and co-production of some toxins (Chen et al., 2015). There is therefore a need to screen for, isolate and characterize more strains of fungi for pigment production. The aim of this work is to screen for, and isolate pigment-producing filamentous fungi, and to evaluate the ability to produce different shades of pigments on PDA under various culture conditions. In the present report, the authors described the isolation, molecular identification and investigation of pigment production potentials of *Talaromyces purpurogenus* isolated from soil sample collected from cassava processing site using solid state cultures.

MATERIALS AND METHODS

Fungi isolation

All the media components used in this study, except otherwise stated, were obtained from Wako Pure Chemical Industries Ltd, Japan. Soil samples were collected from various environments where cassava (*Manihot esculenta* Crantz) tubers are processed into a local staple called 'garri', rice mills, potato farms and corn fields in Eastern part of Nigeria. The soil samples were serially diluted with sterile distilled water and plated out on potato dextrose agar (PDA) in Petri dishes containing 5 µg/ml of chloramphenicol (Pfizer Pharmaceuticals). The plates were incubated at room temperature (25 ±3°C) for seven days. Colonies that showed some pigmentation were picked and sub-cultured in freshly prepared PDA Petri dishes. The sub-culturing was done several times to obtain pure cultures of fungi with high pigment production potential.

Morphological identification of the fungal isolate

The fungus was inoculated at three points in 9 cm Petri dishes on PDA (Difco) and incubated at 25±3°C for seven days. The isolate was identified by examining the colony morphology, microscopic observation of the hyphae, conidiophores and conidia using light microscope BX51 (Olympus Optical Co., Ltd, Tokyo Japan). Photomicrograph was taken using HK 3.1 CMOS digital camera attached to Olympus BX51 microscope and a scanning electron microscope (LEO Model 1450VP Variable Pressure Scanning Electron Microscope Carl Zeiss, Cambridge, MA, USA).

DNA isolation, amplification and sequencing

Total genomic DNA was extracted from the isolate using the method

of Manmur (1961). The internal transcribed spacer region (ITS) ITS-5 and 4 were amplified using the method of White et al. (1990). The primers used were Prime STAR HS DNA polymerase (TakaraBio, Japan). The amplified PCR product was sequenced using BigDye Terminator v 3.1 cycle sequence kit (Applied Biosystems, CA, USA) while the sequencing was done using ABI PRISM 3130 X 1 Genetic Analyzer System (Applied Biosystems, CA, USA). The sequence was compared with reference ITS sequence from GenBank at DDBJ/EMBL, using Apron DB-FU 6.0 (Technosuruga Laboratory Co Ltd, Japan). The nucleotide sequence was deposited in Genbank (DDBJ/EMBL) and was assigned the accession number LC128689. Phylogenetic relationships were analyzed by molecular evolutionary genetic analysis using Apron 2.0 software (Technosuruga Laboratory Co Ltd, Japan). A neighbour-joining tree was constructed using the ITS-5.8S rDNA. Bootstrap analysis was performed with 1,000 replications to determine the support for each clade.

Sub-culturing and storage of the isolate

The cells were sub-cultured in PDA (39 g/L) test tube slants, incubated at 25°C for seven days and stored in a refrigerator at 4°C. The sub-culturing was done once every six weeks.

Pigment production potentials of the newly identified T. purpurogenus isolate

This was investigated in solid state cultures using PDA in Petri dishes. Each experiment was performed three times and the average values plus/minus the standard error of the means were plotted. Effects of the following parameters on pigment production were investigated namely: Plating methods (surface plating, pour plating, pour plating with agar overlay, thickness of agar overlay), spore inoculum concentration, illumination and temperature.

Effect of plating methods on pigment production by T. purpurogenus isolate

PDA (39 g/L) was dispensed at 25 ml per Petri dish after autoclaving. The active *T. purpurogenus* spore suspension (0.1 ml) containing 1.5×10^7 spores /ml was used to inoculate the surface of the agar plates and spread out using a glass spreader. In the case of pour plating, the autoclaved PDA (25 mL) in test tubes were left to cool to about 45°C and each test tube was inoculated with 1 ml of 1.5×10^6 spore suspension, vortexed briefly to mix and quickly poured into sterile Petri dishes. The plates were prepared in triplicates. After solidification, one set was overlaid with (10 ml) of PDA medium and the edge was taped round with paper tape. The other set was taped round with paper tape without any agar overlay. The three sets of differently treated plates were incubated at 25°C for 96 h. Effects of thickness of the agar layer was also investigated by pouring 10 mL or 15 mL of agar on top of solidified inoculated PDA, resulting respectively to 2.66 and 4.00 mm thick un-inoculated PDA on top of seeded PDA in Petri dishes. All other culture conditions were as described before.

Effect of spore inoculum concentration on pigment production by T. purpurogenus isolate in solid state culture

PDA at 39 g/L was prepared and dispensed in 25 ml aliquots into test tubes as described before. The test tubes were sterilized by autoclaving at 121°C for 15 min. After cooling to 45°C each test tube was then inoculated with one of the following spore inoculum concentrations 2×10^6, 2×10^7, 2×10^8 or 2×10^9 per test tube. Each

test tube was prepared in triplicates and poured into Petri dishes as described before. The surface of each Petri dish was overlaid with 15 ml of sterile PDA and taped round with paper tape. The Petri dishes were incubated at 25°C for 96 h.

Effect of light illumination on pigment production by T. purpurogenus isolate

Pour plate with 15 ml of sterile PDA over laid on top and sealed round with paper tape were prepared in triplicates and one set was incubated in the dark and the other under white unscreened illumination using white fluorescent lamp. The light intensity was measured with lux meter (Yokogawa digital lux meter 51011 series Japan).The inoculum spore concentration was 2×10^8 spores per Petri-dish, the incubation time and temperature were 96 h and 25°C, respectively while the light intensity was 50 µmol/m²·s (1 lux = 0.0185 µmol/m²·s).

Effect of temperature on pigment production by T. purpurogenus isolate

Pour plates with seal were prepared in triplicates as described previously. One set was incubated at 25°C and the other at 30°C. The inoculum spore concentration was 2×10^8 spores per Petri-dish while incubation was carried out in the dark for 96 h.

Pigment extraction for spectrophotometric determination of the unit optical density

At the end of each cultivation, a disk of the PDA (about one gram) was cut from three points in a Petri dish using a sterile cork borer. Each disk was accurately weighed and placed in 10 ml of either water or methanol in a test tube. The pigment was left to extract overnight with shaking at 100 rpm at room temperature 25±3°C. After that, the extract from each disk was filtered and the filtrate was used for measurement of the pigment unit optical density per gram of agar (UOD/g). Extract from un -inoculated PDA was used as a blank.

Spectrophotometric determination of pigment optical density

The yellow, orange and red pigments were measured at wave lengths of 400, 460 and 500 nm respectively using UV visible spectrophotometer (Shimadzu Model UV-1200) according to the method of Cho et al. (2002). Filtrate from sterile PDA was used as a blank.

Statistical analysis

Data were subjected to one way analysis of variance (ANOVA) and where significant differences were observed, the means were separated by the Least Significant Difference (LSD) (P = 0.05) test.

RESULTS

Morphological characteristics of the isolate on PDA culture

The surface view (obverse) and the bottom view (reverse) of the isolate after cultivation on PDA for 7 days

Figure 1. PDA plates showing the colonies of the isolate. The isolate was cultivated in PDA at 25°C for 7 days. A: Surface (obverse); B: Bottom (reverse) views. C: Micrograph of the isolate, showing the conidiophores and conidia.

at 25°C are shown in Figure 1A and B, respectively. The fungus produced whitish colonies on PDA plates within the first three days of incubation at 25°C but turned light brown with increase in incubation period. As revealed by the electron micrograph of the reproductive morphology of the newly isolated *T. purpurogenus* (Figure 1C), the conidiophores bear numerous ellipsoidal conidia on the secondary phialides.

Molecular identification of the isolate

Based on the Apron DB-FU 6.0 BLAST, the relationship of the ITS-5.8S rDNA base sequence of the isolate with some related fungal species in the data base is shown in Table 1. All the *Talaromyces* strains in the database showed more than 98.8% base sequence similarity to the new isolate. The neighbour-joining tree of the ITS-5.8S rDNA region showing phylogenetic placement of the isolate is shown in Figure 2 where T denotes Ex-type strain while NT denotes Ex-neotype strain while numbers at the branching nodes represent bootstrap values. On

the basis of the morphological characteristics and base sequence similarity, the isolate was identified as *T. purpurogenus* (Stoll) (Samson et al., 2011).

Effect of plating method on pigment production by *T. purpurogenus* isolate

The results of three plating methods namely: Surface plating, pour plating without and with agar layer on the surfaces are shown in Figure 3A and B. Pour plating with agar layer on top produced the highest pigment (UOD) among the three plating methods examined. Using the pour plating with agar overlay method, the amount of red pigment (1.73 UOD/g) was significantly higher than the orange (1.05 UOD/g) and yellow (1.03 UOD/g) pigments ($P<0.05$) but there was no significant difference between the amounts of orange and yellow pigments ($P>0.05$). Under pour plating without agar overlay, the red, orange and yellow pigments were 0.36, 0.29 and 0.39 UOD/g of agar respectively. Surface plating produced the least UOD of the three pigments viz: 0.15, 0.11 and 0.18 of

Table 1. Comparison of the ITS-5.8S rDNA Base Sequence of the isolate with some related fungal species in the database.

Strain	Code	Accession No.	Sequence similarity (%)
Talaromyces flavus	XSD-46	EU273527	562/564 (99.6)
T. flavus	SP5	JF509738	559/562 (99.5)
T. flavus	wb239	AF455513	560/564 (99.3)
T. flavus	wb252	AF455509	559/562 (99.3)
T. Purpurogenus	CBS108923	JX965236	561/563 (99.6)
T. purpurogenus	CBS113158	JX965235	561/563 (99.6)
T. purpurogenus	CBS113161	JX965234	561/563 (99.6)
T. purpurogenus	DTO193H5	JX965233	561/563 (99.6)
T. purpurogenus	DTO189B4	JX965231	561/563 (99.6)
T. funiculosus	UOA/HCPF<GRC>:13814	KC254067	560/562 (99.6)
T. purpurogenus	DTO173E6	JX965230	560/563 (99.5)
T. purpurogenus	DTO189A1	JX315661	541/543 (99.6)
T. purpurogenus	CBS122434	JX315663	541/543 (99.6)
T. funiculosus	NG_p14	HQ115695	541/543 (99.6)
T. purpurogenus	DTO193H1	JX965232	538/540 (99.6)
T. purpurogenus	CBS184.27	JX315665	540/543 (99.4)
T. purpurogenus	CBS286.36T	JN899372	556/563 (98.8)
Penicillium sp.	WD02	KC678995	526/531 (99.1)

red, orange and yellow, respectively. Statistically, there was no significant differences in the UOD of all the pigments produced under both pour plating without agar layer and surface plating (P >0.05).

Effect of pour-plating with agar layer on sporulation and pigment production under sealed condition

The results of these two plating methods are shown in Figure 4. Pour plating, with agar overlay and sealed edges produced significantly higher UOD/g of the three pigments (red, orange and yellow) than plates without agar overlay with sealed edges (P<0.05). The average pigment UOD/g were 1.13, 0.71 and 0.64 UOD/g agar for red, orange and yellow pigments respectively in plates with agar overlay and sealed edges. Furthermore, the red pigment UOD was significantly higher than those of orange and yellow (P<0.05) under this plating method. Plates without agar overlay but with sealed edges produced 0.33, 0.26 and 0.34 UOD/g which were about 3.38, 2.75 and 2.00 times respectively, lower than the values obtained under pour plates with agar layer and sealed edges. In the case of sealed plates without agar overlay, there was no significant difference in the UOD of red, orange and yellow pigments.

Effect of the thickness of the agar overlaid on top of pour plates on pigment production

The results of the effects of thickness of agar layer (4.00 and 2.66 mm) on pigment production are shown in Figure

5. Thick agar (4.00 mm thick) overlay produced significantly higher UOD of red, orange and yellow pigments than using thin (2.66 mm) agar layer (P<0.05). With thick agar layer, 1.45 UOD/g agar of red pigment was produced against 0.40 UOD/g produced when thin agar layer was used. For orange and yellow pigments, 0.95 and 0.96 respectively were produced by using thick agar layer against 0.30 and 0.38 produced when thin agar layer was used.

Effect of spore inoculum concentration on pigment production

When the optimum plating method of pour plating with agar overlay and sealed edges was confirmed to be the best, the optimum spore inoculums concentration was investigated. The results of the effects of spore inoculum concentration per Petri dish on pigment production are shown in Figure 6. The pigment UOD increased with increase in inoculum concentration up to a certain level. Spore inoculum concentration of 2×10^8 spores/Petri dish was the optimum for production of the red, yellow and orange pigments. The amount of pigments produced with spore inoculums concentration of 2×10^8 spore/plate was significantly higher than those produced with other spore inoculum concentrations tested (p < 0.05).

Effect of illumination on pigment production

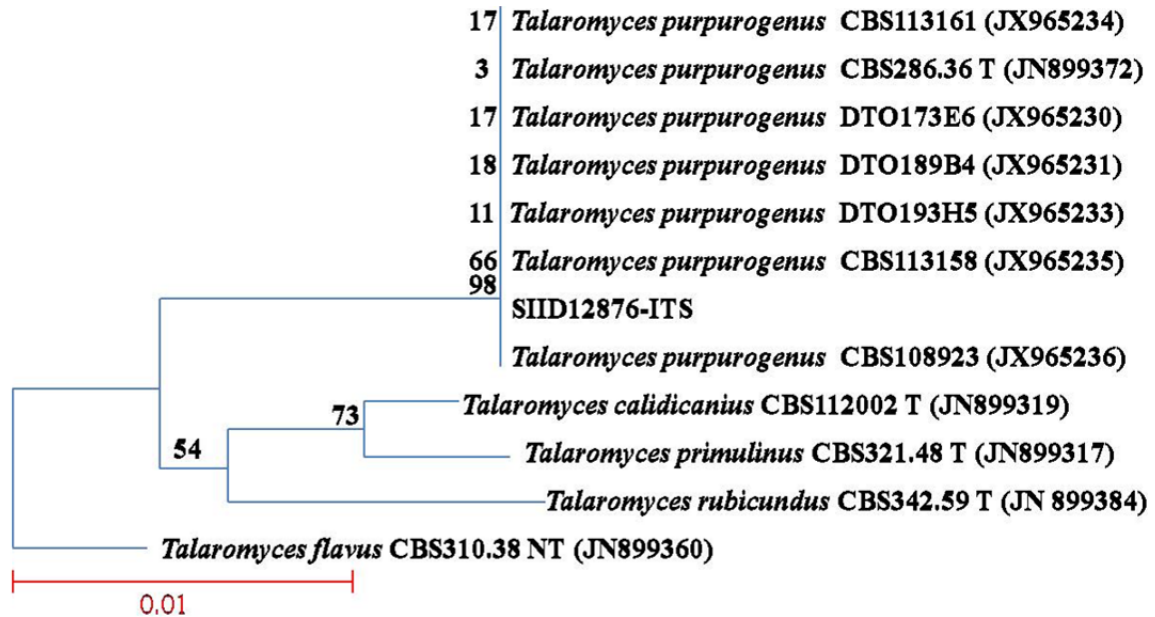The results of the effects of light illumination on pigment

Figure 2. Neighbour-joining tree of the ITS-5.8S rDNA region showing phylogenetic placement of the isolate. T denotes Ex-type strain while NT denotes Ex-neotype strain. Numbers
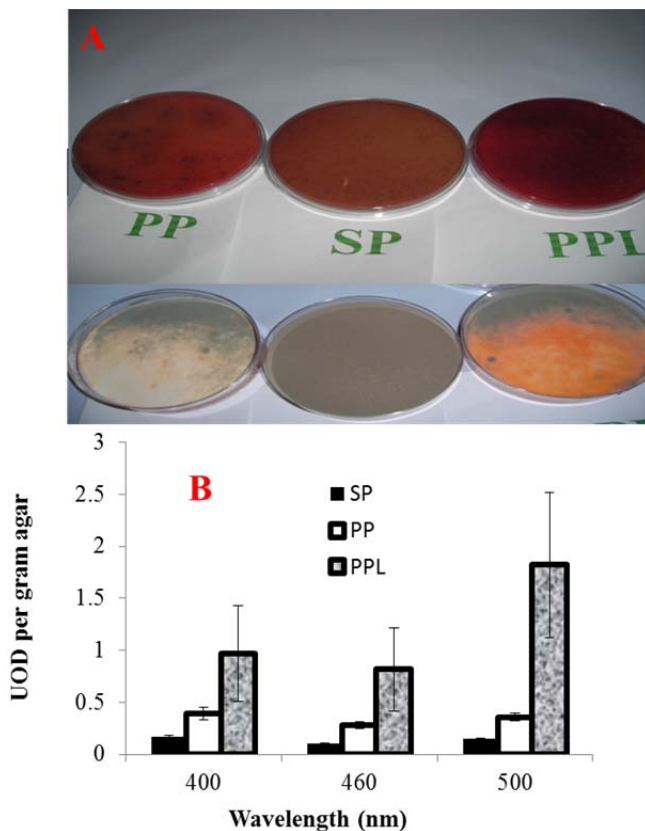

Figure 3. Effect of plating method on pigment production by *T. purpurogenus.* SP, Surface plating; PP, pour plate method; PPL, pour-plate with agar layer. (A) Top and Bottom views of pigments in agar plates after 96 h of cultivation. (B) Data presented are the means ± SE (n = 3). Wavelengths of 400, 460 and 500 nm denotes yellow, orange and red pigments, respectively.

production are shown in Figure 7. Production of red, orange and yellow pigments were similar when grown in the dark and under light conditions. The UOD of red pigments were 5.83 and 5.64 UOD/g under dark and light conditions respectively. Also the UOD of orange and yellow pigments were 4.12 and 4.74; and 3.91 and 4.63 UOD/g under dark and light conditions respectively. On the whole, light illumination had no significant effect on the production of the three types of pigments ($P > 0.05$).

Effect of temperature on pigment production

The results obtained after cultivating at 25 and 30°C are shown in Figure 8. Pigment production was better at 30°C than at 25°C. The average pigment concentrations (UOD/g) obtained at 30°C were 11.20, 7.30 and 8.21 for red, orange and yellow pigments respectively. At 25°C the average values obtained were (UOD/g) 4.75, 3.02 and 3.28 for red, orange and yellow pigments respectively. These were about 2.35, 2.41 and 2.50 times lower than what was produced at 30°C for red, orange and yellow pigments respectively. Pigment production was significantly reduced at 35°C (data not shown).

DISCUSSION

Species of *Talaromyces* have been reported to produce various pigments both in suspended and solid state cultures (Frisvad et al., 2013; General et al., 2014). The present study has also shown that a strain of *T. purpurogenus,* isolated from soil sample collected from
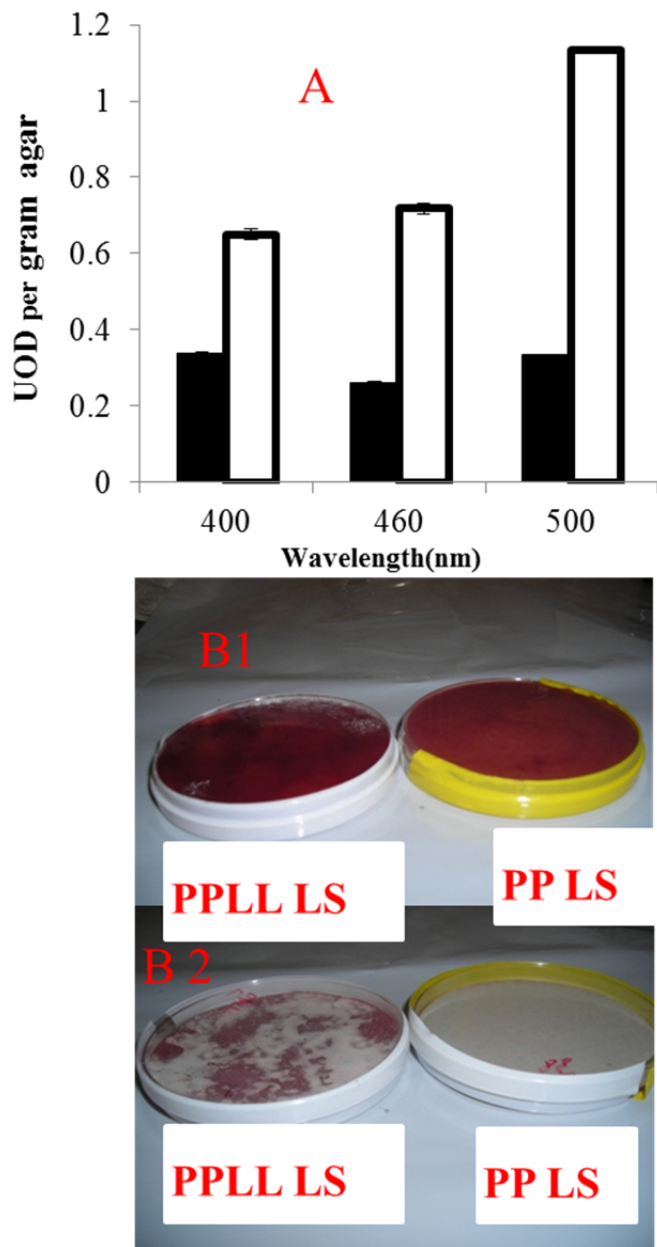
Figure 4A. Effect of pour-plate method with agar layer on sporulation and pigment production under sealed condition. PPLL LS, Pour plating with agar layer (white bar); PP LS, Pour-pating without agar layer (black bar). Figure 4B 1 and 2 are bottom and surface views of pigments in agar plates with the two plating methods after 96 h of cultivation. Wavelengths of 400, 460 and 500 nm denotes yellow, orange and red pigments, respectively. Data presented are the means ± SE (n = 3).

cassava processing site is capable of producing diffusible red, orange and yellow pigments in agar cultures under various conditions. These pigments have potential applications as colouring agents in textile, cosmetics, pharmaceutical, food, and wood industries. The advantages of using solid state culture in pigment

production include the possibility of using cheap substrates, lower cost of purification since contamination by media components is minimal, and also generation of less effluent (Babitha et al., 2006; Kapilan, 2015).

Plating method has a profound effect on metabolite production by fungi. With surface plating, there was profuse hyphal growth and sporulation with less pigment. This might be because of high aeration and evaporation rate in Petri dishes. Pour plating method is an effective method of cultivating fungi for metabolite production and it is interesting to note that production of all the three pigments was higher in pour-plate method than in surface plating. With pour plating, the spores were more evenly distributed on the agar plates which reduced competition for nutrients. It is also very interesting to observe that pour plating with an agar overlay and sealed edges produced the highest quantity of pigments among surface plating and pour plating without agar overlay. Overlaying the agar surface inside Petri dish with agar insulated the organism, conserved the moisture contents, reduced aeration and thus prevented profuse vegetative growth thereby channelling all the energy to pigment production. Sealing the Petri dishes with paper tape also provided additional insulation and prevented much water loss by evaporation from the culture. This seems to be the first report on using this technique of pour plating with agar overlay for pigment production by a fungus. This knowledge will be useful in controlling the moisture content/humidity when grains and other solid substrates are used for large scale metabolite production.

Spore inoculum concentration is an important parameter in any fermentation process because too high spore inoculum will deplete the nutrient for vegetative growth soon after inoculation. Under this condition, there will be little or no nutrient for metabolite production. On the other hand, too little cell inoculum will result in sluggish growth and inefficient nutrient utilization. Velmurugan et al. (2010a) also reported that spore inoculum concentration of 4 ml containing 6×10^5 spores/ml was the best for inoculation of 5 g of substrate for solid state fermentation. General et al. (2014) reported optimum inoculum volume of 1.8×10^6 spores per gram solid substrate during pigment production by *Talaromyces amestolkiae* using macroalgal biomass as the substrate.

Fungi, respond to light during growth and metabolite production just like most prokaryotes and eukaryotes. Our results showed that pigment production was favoured more by incubating in a dark place than under light. The effects of light on pigment production by fungi have been studied by many researchers. Buhler et al. (2015) reported that during cultivation of *M. ruber*, growth and pigment production were inhibited in Petri dishes and baffled flasks exposed to direct illumination. Velmurugan et al. (2010b) also noted that growth and pigment production by *M. purpureus, Isaria farinosa*, *Emericella nidulans*, *Fusarium verticillioides* and *P. purpurogenum* were higher under dark condition than when exposed to
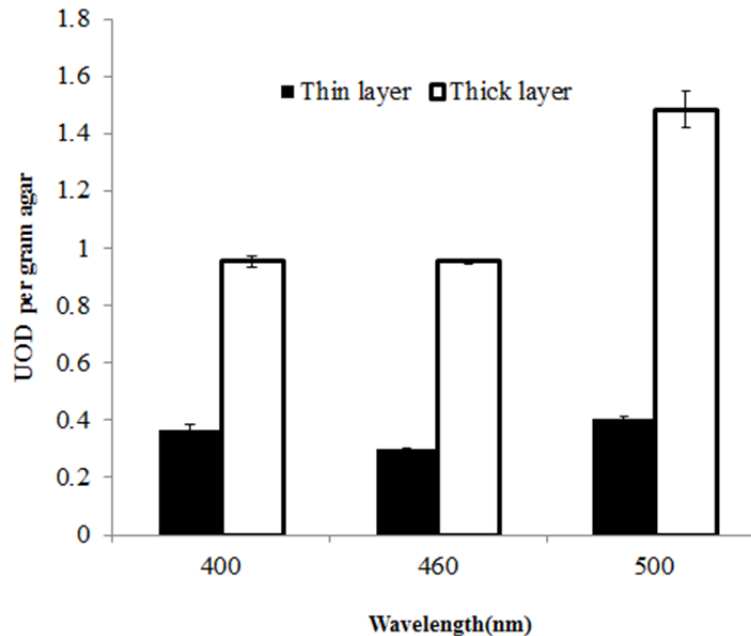
Figure 5. Effect of the thickness of agar overlay on pigment production. The thickness of the thin layer was 2.66 mm while that of the thick layer was 4.0 mm. This was done by pouring 10 ml and 15ml of un-inoculated PDA respectively onto seeded solidified potato dextrose agar in Petri dishes. Wavelengths of 400, 460 and 500 nm denotes yellow, orange and red pigments, respectively. Data presented are the means ± SE (n = 3).
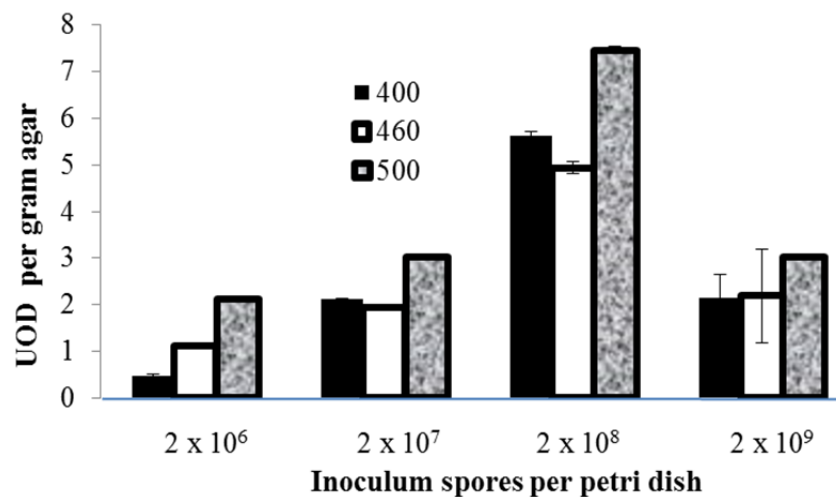

Figure 6. Effect of spore inoculum concentration on pigment production.Three levels of spore concentration were used to inoculate 25 ml of autoclaved PDA in test tubes. The inoculated PDA was then poured into Petri dishes, overlaid with 15 ml of agar after solidification, taped round and incubated at 25°C for 96 h. Wavelengths of 400, 460 and 500 nm denotes yellow, orange and red pigments, respectively. Data presented are the means of ± SE (n = 3).

lights of various wavelengths. In the case of *M. purpureus*, Velmurugan et al. (2009) reported that incubation in total darkness increased red pigment production but illumination resulted in total suppression of pigment production.

Most fungi are mesophiles whose growth and metabolite production occur mostly within the temperature range of 25 and 30°C. The optimum
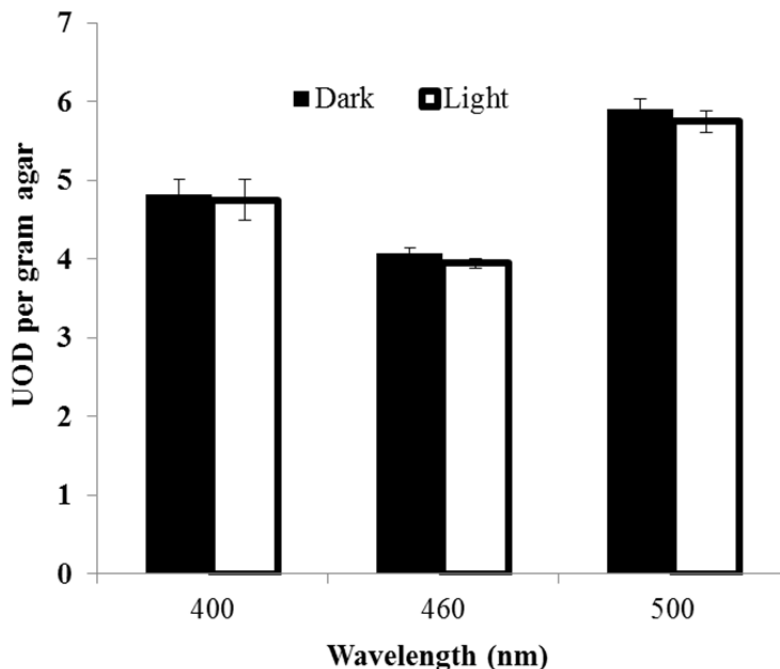
Figure 7. Effect of illumination on pigment production. PDA plates were prepared in triplicates by pour plating. The plates were inoculated with 2×10^8 spores/ mL taped round. One set was incubated in dark and the other under illumination with light intensity of 150 micro mol/m^{2}s. Wavelengths of 400, 460 and 500 nm denotes yellow, orange and red pigments, respectively. Data presented are the means of ± SE (n = 3).
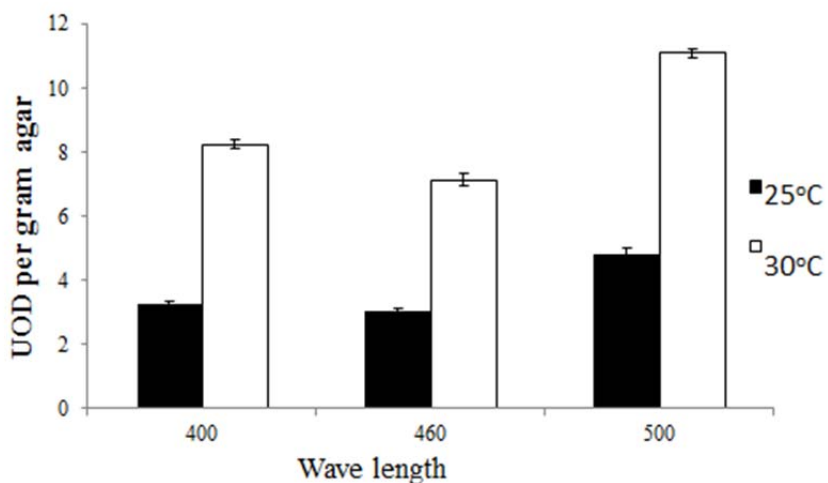

Figure 8. Effect of temperature on pigment production by *T. purpurogenus.* Pour plates with agar overlay and seal were prepared in triplicates. One set was incubated at 30°C and the other set was incubated at 25°C. The inoculum concentration was 2×10^8 spores per Petri-dish while incubation was in dark. Wavelengths of 400, 460 and 500 nm denotes yellow, orange and red pigments, respectively.

temperature for pigment production in solid state culture in Petri dish by this isolate of *T. purpurogenus* was 30°C. This was in agreement with the results of pigment production by *Penicillium aculeatum* ATCC 10409 (Afshari et al., 2015), *M. ruber* (Said et al., 2010) and *M. purpureus* CMU001 (Nimnoi and Lumyong, 2011). With

all these species, the optimum temperature was reported to be 30°C. When the newly isolated *T. purpurogenus* was cultivated at 35°C there was good growth but pigment production was very low.

Under the present experimental conditions, red, orange and yellow pigments of 11.2, 7.28 and 8.21 UOD per gram of wet agar were obtained. This is comparable with the results of other researchers who used dried fermented substrates considering the water content of the substrate in the present study. For example, Velmurugan et al. (2009) reported 30.8 and 25.5 absorbance unit per gram of dry fermented substrate for red and yellow pigments respectively using jackfruit seed supplemented with monosodium glutamate as substrate. Pigment production by the newly isolated *T. purpurogenus* in suspended liquid culture under various culture conditions is currently under investigation. On the whole, the above results have shown that pigment production by *T. purpurogenus* is highly affected by temperature, inoculum concentrations and plating methods.

Conclusion

A pigment producing *T. purpurogenus* was successfully isolated from the soil and identified based on the macroscopic and microscopic morphology and the ITS-5.8S rDNA base sequence. All the *Talaromyces* strains in the database showed more than 98.8% base sequence similarity to our new isolate. The optimum plating method for pigment production by this isolate was pour plating with thick agar layer of 4 mm on top and sealed edges. The optimum spore inoculum concentration was 2×10^8 per Petri dish. Pigment production was not affected by illumination conditions. The unit optical density of pigments produced at 30°C was higher than the values obtained at 25°C. The results of this study have revealed that the new isolate of *T. purpurogenus* has high potentials for industrial pigment production.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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