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Growth and pigment production of *Monascus purpureus* EG and its beneficial effects

Amira A. El-Fallal✉, Aya Maher Abo-Zied, Mohamed M. El-Zahed

Department of Botany and Microbiology, Faculty of Science, Damietta University, New Damietta 34517, Egypt

✉Corresponding author: *aelfallal55@gmail.com*

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Abstract

Pigments play an important role in the pharmaceutical industry as well as in the food industry. Biological synthesis of pigments has attained more revenue for easy extraction, high growth rate and high yield. The production of pigments by *Monascus purpureus* EG was investigated in several static batch cultures, the most suitable medium for its yellow, orange, and red extracellular pigment production was yeast glucose medium (YG), while malt extract medium (ME) had maximum production for its intracellular pigment. The effect of some physical factors on growth and pigment production was studied. Direct illumination inhibited growth and pigment production. Antimicrobial activities of pigments were observed against selected Gram-negative (G-ve) and Gram-positive (G+ve) bacteria. The antibacterial effects of red pigment on G- and G+ were highly effective compared to yellow and orange pigments. The extracted pigment was used for the reduction of the aqueous silver nitrate into silver nanoparticles (AgNPs). The biosynthesized AgNPs were structurally characterized using UV-VIS Spectra which showed absorption peaks at 437, 453 and 447 nm for pH values 3.5, 6.5 and 9.5, respectively. The optimum pH for the maximum synthesis of nanoparticles was 6.5. It showed no nitrate reductase activity, but the synthesized AgNPs exhibited strong antimicrobial activity against G+ and G- bacteria.

Introduction

Monascus purpureus belongs to the phylum Ascomycota, Monascaceae family is purplish-red in shading. For quite a while, it has been customarily significant in China, Thailand, and Japan for the creation of red rice wine, red soybeans, cheddar, and ang-kak rice. It was discovered to create safe regular colours and other remedially significant auxiliary metabolites, including citrinin and statins (Liu *et al*. 2020). It is not just utilized as a food colorant, seasoning specialist, and additive; however, it is likewise generally applied in clinical purposes to bring down blood cholesterol, hostile to diabetes, mitigating and to forestall osteoporosis (Arunachalam and Narmadhapriya 2011). It has the capacity as a saccharification specialist and ethanol maker. The past examination by Takeshita *et al.* (2016) indicated that a mix of *M. purpureus* and *Saccharomyces cerevisiae* K7 could create ethanol in mixed drinks. The shade of a food substance is essential to mirror its newness and security. It is likewise a pointer of good tasteful and sensorial qualities (Malik *et al*. 2012).

Ongoing years, the food business has zeroed in on creating characteristic colours from plants and microbial sources to defeat the utilization of manufactured shades that are possibly dangerous to human well-being and the climate. Regular colours delivered by microorganisms have acquired significance due to their low water solvency and the shaky idea of plant inferred shades against warmth and light (Sharmila *et al*. 2013). The main trait of *M. purpureus* is its capacity to integrate colours from polyketide chromophores and β-keto acids by esterification. The shades created by it are grouped into in any event six sorts of shades dependent on shading: (1) red colour (rubropunctamine and monascorubramine); (2) orange colour (rubropunctatin and monascorubrin) and (3) yellow colour (monascin and ankaflavin) (Fig. 1) (Guo *et al*. 2016). The structure of colours created by *Monascus* species relies upon elements, for example, the sort of substrate and nitrogen source, pH, and temperature (Haque *et al*. 2016).

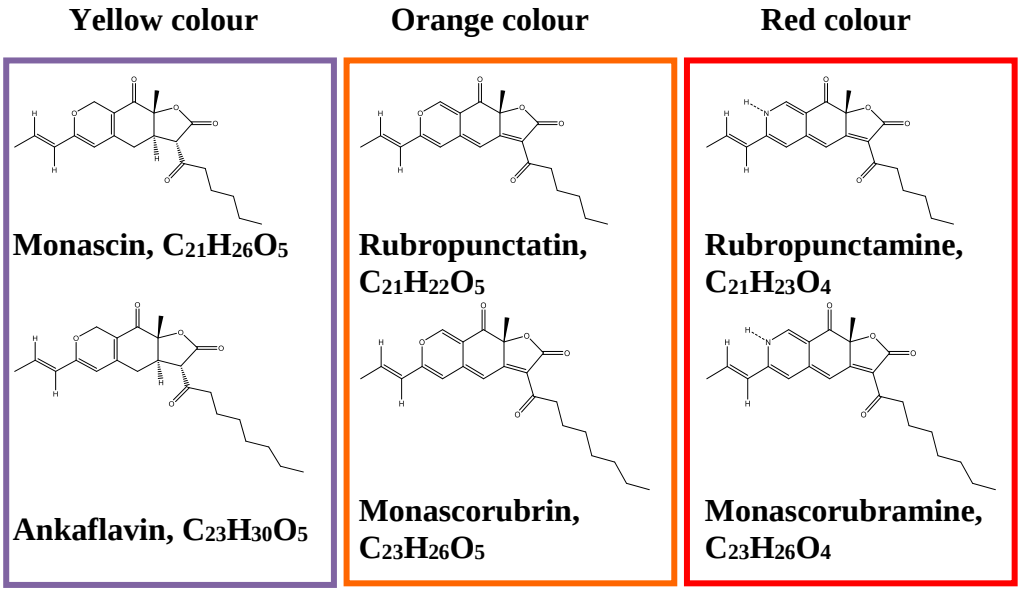


Fig. 1. Six major *Monascus* pigments chemical structures.

Current exploration found that *Monascus* shades (MPs) had a scope of organic exercises, for example, cell reinforcement, anticancer properties, anti-cholesterols, and invulnerable enhancer metabolites (Park *et al*. 2005; El-Sayed *et al*. 2020). As the *Monascus* can produce lovastatin alongside with function as a colour it is also a cholesterol-lowering specialist by stifling cholesterol amalgamation using hindrance of HMG-CoA reductase compound, which intervenes the biosynthesis of cholesterol in the liver and is known as a strong inhibitor simvastatin (Seenivasan *et al*. 2020). In this manner, the presence of lovastatin in bioproducts of a life form is an extra preferred position making *M. purpureus* significant in clinical and drug ventures by agreeably working with different mixes or independently to animate body cells in the therapy of Alzheimer's infection or malignant growth, enlistment of apoptotic cell demise, a decrease of aggravation, hindrance of tumorigenesis and viral replication (Choe *et al*. 2020).

The present study aims to evaluate the best possible fermentative conditions for maximum production of bio-pigment, its antimicrobial effects and study *M. purpureus* pigments as a reducing and capping agent for the rapid synthesis of silver nanoparticles (AgNPs).

Experimental

Microbial cultures

M. purpureus EG was obtained from the Microbiology Lab (Mayada El-Fawal), Botany and Microbiology Department, Faculty of Science, Damietta University, Egypt. It was maintained on Potato Dextrose Agar (PDA) medium at 30 ºC and preserved at 4 ºC, with sub-culturing every four

weeks. The bacterial strains (*Bacillus subtilis* and *Escherichia coli*) were kindly provided by Prof. Mohamed I. Abou-Dobara (Professor of Microbiology, Botany and Microbiology Department, Faculty of Science, Damietta University, Egypt).

Chemicals

Silver nitrate was purchased from Panreac Quimica S.L.U (Barcelona, Spain). The other chemicals and solvents were purchased from Sigma Aldrich (Saint Louis, USA) and El Nasr Pharmaceutical Chemicals Company (Oubour, Qalyubi, Egypt).

Cultivation media

Yeast glucose medium (YG) (Chen and Tseng 1989), Modified Lin's medium (ML) (Lee *et al.* 2001), Sabouraud dextrose medium (SD) (Jacobs and Gerstein 1960), malt extract medium (ME) (Thom and Church 1926), Czapex-Dox medium (Waksman 1967) and potato dextrose medium (PD) (Beever and Bollard 1970) were the basic fermentation media. Nutrient agar medium was used in the study of antimicrobial activity tests.

Evaluation of different media, physical parameters on growth and pigment production

Three different solid culture media potato dextrose agar (PDA), malt extract agar (MEA) and Sabouraud dextrose agar (SDA) were prepared then sterilized by autoclaving, inoculated centrally on Petri dishes containing those media with a 6mm. agar disc cut, with sterilized cork-borer from the edge of 7-day old *M. purpureus* culture growing on PDA agar and incubated for 7 days at 20, 30, and 50 ºC and then after incubation period the most suitable degree of temperature for growth and pigment production was determined. The effect of dark and light was performed in Petri dishes and flasks. *M. purpureus* culture was covered (dark conditions) with aluminium foil paper for comparison with the other culture that was continuously cultured under light for 7 days at 30 ºC.

PD, SD, ME, YG, ML broth media were sterilized at 121 ºC for 15 min then were inoculated with a 6mm agar disc of M. *purpureus* and incubated for 7 days at 30 ºC under static conditions (Memmert incubator, Memmert GmbH + Co. KG, Schwabach, Germany). After incubation, the mycelia were separated from the broth by using pre-dried and weighed Whatman filter No. 1 then was dried in an oven at 80 ºC and weighed to determine biomass.

Extracellular pigments analysis

Estimation in static culture was performed using culture filtrate. The pigment mycelia were separated from broth by filtration. The cell-free culture filtrate was then centrifuged (Xiangshui Fada medical apparatus factory, model 800, Xiangshui FADA Medical treatment machinery Factory, Xiangshui, China.) at 7,511×g for 15 min, and the supernatant was decanted. The filtrate was diluted with distilled water, and the extracellular pigment concentration was determined using a UV-visible spectrophotometer (model UV1100, Shanghai Yoke Instrument Company Co., Ltd., Shanghai, China.) at wavelengths of 400, 460 and 500 nm for yellow, orange, and red pigments, respectively. Distilled water was used as blank control. The amounts of extracellular pigments were obtained via multiplying the sample absorbance by the applied dilution (Yang *et al.* 2019).

Intracellular pigments analysis

The mycelia were washed twice by distilled water and collected, crushed, and soaked in 10 mL of ethanol aqueous solution (70 % v/v; pH = 2) for 2 h in a horizontal shaking incubator (Incubator WIS-10 Shaking incubator top-door orbital motion benchtop shaker incubator, model WIS-10, Witeg Labortechnik GmbH, Wertheim, Germany) at 0.4×g to extract the intracellular pigments. The ethanol extract of intracellular pigments was centrifuged at 7,511×g for 15 min to separate the mycelia, after which the supernatant was collected. The corresponding ethanol aqueous solution was subjected to intracellular pigment concentration measurement using a UV-visible spectrophotometer (Shanghai Yoke Instrument Company, model UV1100, China) at wavelengths of 400, 460 and 500 nm for yellow, orange, and red

pigments, respectively. Ethanol (70 % v/v, cut-off wavelength; 210) was also used as blank control. The amounts of intracellular pigments were obtained by multiplying the sample absorbance by the applied dilution (Yang *et al*. 2019).

Antimicrobial activity of pigments

Extracted pigments were tested for their antimicrobial activity against two types of bacteria by using the agar well diffusion method. The tested microbial strains were *E. coli* and *B. subtilis*. Standardized suspension of each of the tested strains (10^8 CFU.mL^{-1}) was swabbed uniformly into the sterilized culture medium (nutrient agar medium), mixed well, poured to Petri plates and made discs then placed filtrate of both culture media in each disc. The plates were incubated for 24 h at 37 ºC, the inhibition zones were measured.

Silver nanoparticles biosynthesis using Monascus red pigment

The extracellular *Monascus* red pigment-mediated synthesis of AgNPs was executed as follows, 100 µL (1 mg.mL^{-1} concentration) of red pigments solution was added into 2.5 mL silver nitrate ($AgNO_3$) (2.0×10^{-4} M) solution (pH 7.0 ± 0.2) in clean glass tubes. Then, the tube was vigorously shaken for proper mixing of the reactant and kept under high-intensity sunlight and observed for the visual colour change of silver salt solution. Simultaneously, the controls of silver nitrate (2.5 mL silver nitrate without pigments) and pigments (100 µL pigments in water) were separately run at the same condition (Koli *et al*. 2018).

Nitrate reductase activity

Nitrate reductase activity of the fungus was assayed by obtaining two tubes, the first one was mycelia of the fungus with 10 mL of $AgNO_3$ and the second one was the control which consisted of 10 mL of supernatant of the fungal filtrate and 10 mL of water then incubated in shaking incubator (benchtop shaker incubator, model WIS-10, Korea) in the dark conditions for 5 – 7 days until the developed brown colour appeared and then measured by UV-VIS; spectrophotometer at the wavelength of 540 nm.

Characterization of AgNPs

The formation of brown or yellow-orange color after reaction of *Monascus* pigments with whitish color bulk $AgNO_3$ solution in the presence of sunlight was the primary indication of conversion of silver nitrate into nanosized particles.

UV-VIS spectrophotometric analysis

The extracellular red *Monascus* pigment-mediated synthesis of AgNPs was confirmed by scanning of brown AgNPs solution on UV/VIS/NIR spectrophotometer (model V-630, JASCO International Co., Ltd., Hachioji-shi, Japan) in the range of 300 – 700 nm.

Effect of pH on AgNPs synthesis

The optimum concentration of $AgNO_3$ (1 mM) was added to the fungal filtrate and the pH of the reaction mixture was adjusted to 3.5, 6.5, and 9.5 (El-Baz *et al*. 2016), using 1 M HNO_3 and 1 M NaOH solutions to obtain the optimum conditions for the maximum synthesis of nanoparticles.

Antimicrobial activity of AgNPs at different PH values

Biosynthesized AgNPs at different PH values 3.5, 6.5, and 9.5 were tested for their antimicrobial activity against two types of bacteria: *E. coli* and *B. subtilis* by also using the agar well diffusion method and after incubation for 24 h at 37 ºC, the inhibition zones were measured.

Statistical analysis

The data were statistically analyzed using SPSS software version 18. All values in the experiments were expressed as the mean ± standard deviation (SD).

Results and Discussion

Morphological characters of M. purpureus on different media

Different morphological characteristic of *M. purpureus* was shown when grown on different solid culture media such as PDA, MEA and SDA. In the early stages of mycelial growth on Malt and Sabouraud media the mycelium was white and rapidly turned to rich orange with small aerial development and in the case of PDA medium it changed into red color and each colony has whitish edges (Fig. 2).

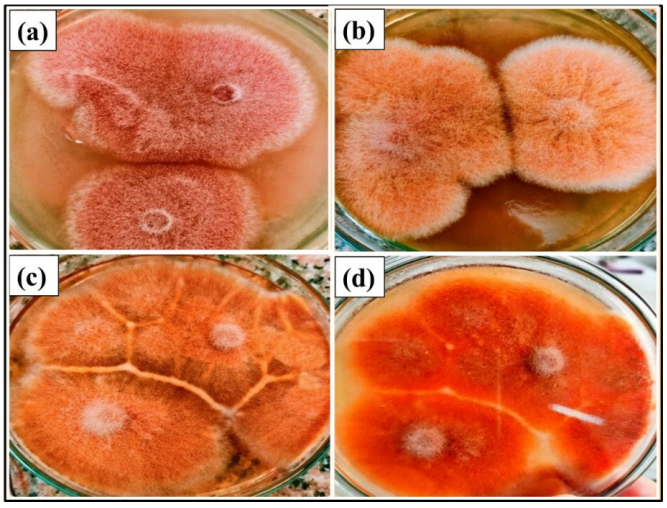


Fig. 2. Growth of *M. purpureus* EG on different solid culture media **(a)** PDA, **(b)** MEA and **(c)** SDA, **(d)** Dorsal view showing pigment production on SDA. Based on microscopic examination we observe that is the formation of cleistothecium that contains up to 120 or more oval ascospores which means that *M. purpureus* preferred sexual reproduction (Patrovsky *et al*. 2019).

Effect of temperature on M. purpureus growth and pigment production

The results showed that *M. purpureus* grew in two tested temperatures (20 ºC and 30 ºC) and was not able to grow at 50 ºC. The maximum growth and pigmentation were observed at 30 ºC (Fig. 3). It was shown less growth and pigmentation at 20 ºC. The activity of enzymes for red pigment production was optimum at 30 ºC, but it stopped at 50 ºC. It can be concluded that temperature plays a vital role in the metabolism of cells, as it affects the growth and pigment yield. Carvalho *et al*. (2005) also reported a shift in absorbance maxima of pigment yield at different incubation temperatures.

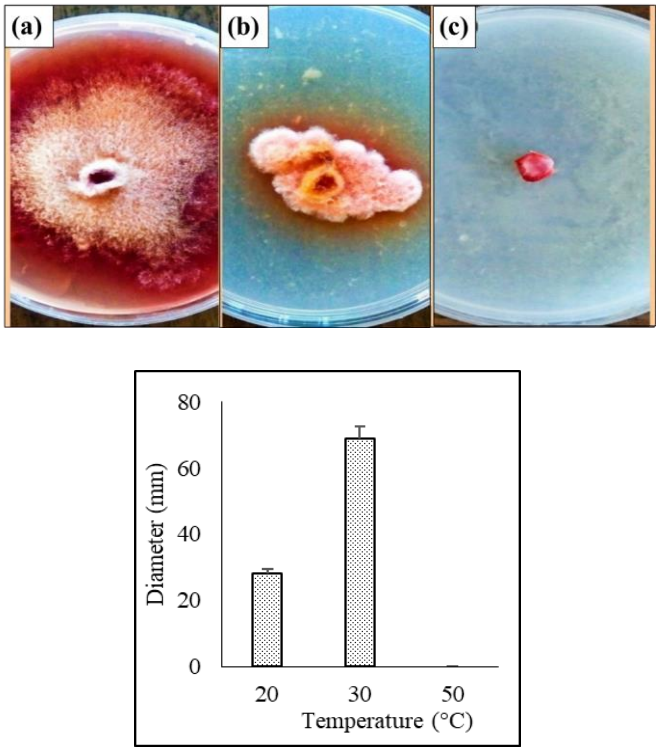


Fig. 3. Effect of temperature on growth and pigment production of *M. purpureus* **(a)** 30 ºC, **(b)** 20 ºC and **(c)** 50 ºC.

Effect dark and light conditions on M. purpureus growth and pigment production

In this study, it is observed that the production of pigment increased by the incubation in total darkness and there was a reduction in growth and pigment yield under continuous illumination. These assays were performed on different culture mediums (Fig. 4). There was an increase in the biomass of *M. purpureus* in total darkness and a clear reduction in continuous illumination and these observations disagree with Babitha *et al*. (2008) who reported that when considering the growth of the organism, direct illumination favoured the growth but under total darkness, there was a reduction in the biomass.

The wavelength dependence of this response was investigated for red pigment production, and it had shown that light inhibits the pigment yield as in the case of total darkness the absorbance was 2.209 and under continuous illumination was 1.371. Colonies with more growth were observed in Petri dishes covered with aluminium foil, and linear extension was determined (Table 1).

Table 1. The diameter of *M. purpureus* radial growth (mean ± SD) on different culture media in dark conditions and the presence of light.

Culture media	Diameter [mm]	
	Dark	Light
MEA	90 ± 0.2	50.8 ± 0.2
SDA	70 ± 0.5	50.1 ± 0.4
PDA	70 ± 0.1	30.6 ± 0.2

Light has a depression influence on spores and aerial mycelium production, which was similar to Zhang *et al*. (2020) results. The negative effect of light can be reversed by applying darkness exposure.

Miyake *et al*. (2005) studied the effect of different conditions of illumination, using the blue, green, and red light and he found that the red light showed little effect on the growth and pigment production.

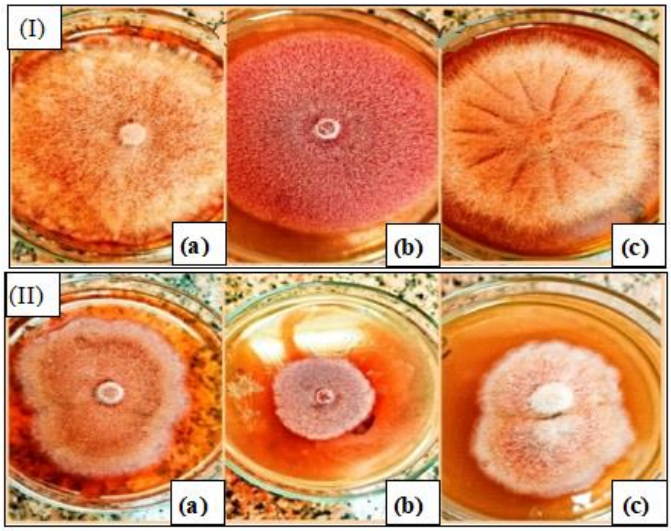


Fig. 4. The effect of light; (I) and dark; (II) conditions on *Monascus* culture morphology and pigment production while growth on **(a)** MEA, **(b)** PDA and **(c)** SDA.

The colonies grown under the direct illumination resulted in less pigment production and that may be according to the presence of photoreceptors that make a response to dark and light like these receptors that found in *Neurospora crassa* as reported by Ballario and Macino (1997).

Effect of different liquid culture media on growth and pigment production

The results showed that *M. purpureus* grew rapidly on all tested culture media, but the texture of mycelium and color was dependent on media type (Fig. 5). We observed that PD and ME were the best media to promote fungal growth after incubation for 7 days at 30 ºC. The highest dry weight was achieved when *M. purpureus* grew on PD medium (Fig. 6). These results indicated that this fungus has a complex system of the enzyme (lytic enzymes) that enables it to grow on different substrates and we can say that it preferred growth on complex culture mediums.

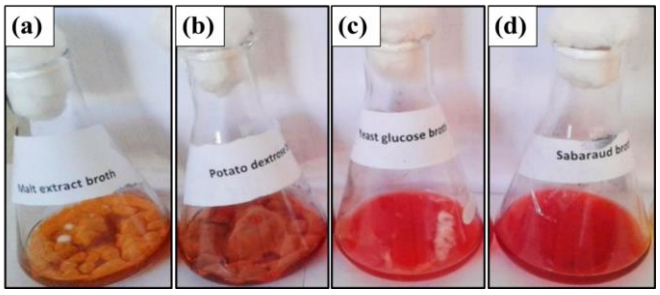


Fig. 5. Batch fermentation profile showing pigment production and biomass of *M. purpureus* while growth on **(a)** ME, **(b)** PD, (c) YG and **(d)** SD broth media.

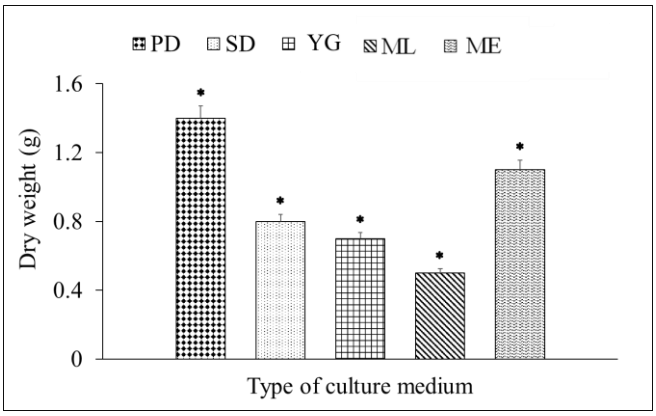


Fig. 6. The effect of different culture media on *M. purpureus* mycelial dry weight (g). *M. purpureus* grow on the PD medium better than ML medium followed by SD, YG and ML broth media, respectively (Highly significant = * P<0.05, n = 3).

The current study focused on determining intracellular and extracellular yellow, orange, and red pigments in different culture media. Fig. 7 showed that the YG broth medium was the best culture medium for maximum production of extracellular yellow, orange, and red pigments of 1.591, 1.912, and 2.391, respectively. This current result was similar to Pisareva and Kujumdzieva

(2010) and Lee *et al*. (2001) who concluded that glucose as the sole carbon source was suitable for producing high pigment yields. The mechanism for high pigment yield may be related to the high expression levels of certain proteins in the polyketide synthesis pathway and the high concentration of primary metabolism-generated molecules as substrates for polyketide synthesis (Huang *et al*. 2018). ME broth medium had the maximum production of intracellular yellow, orange, and red pigment of 1.508, 1.869 and 2.377, respectively. Our finding is in agreement with Huawei *et al*. (2019) who reported that maltose was the most suitable carbon source responsible for high intracellular *Monascus* pigments.

Patrovsky *et al*. (2019) studied the influence of initial pH and nitrogen source on the production of *Monascus* pigments during submerged liquid cultivation and reported that red pigments are made by the reaction of orange pigments with compounds containing an amino group, and the reaction is followed by the formation of a mixture of red pigment.

On the other hand, Talapragada *et al*. (2017) revealed that specific amino acids at 1% influence the type and amount of extracellular or intracellular bio-products and fresh biomass produced by *M. purpureus* under submerged static conditions. Pengnoi *et al*. (2017) reported that pigment production in this fungus can be achieved by solid-state fermentation technique using agricultural products other than rice. Cornmeal supplemented with 8 % (w/w) glucose was a suitable substrate for pigment production. So, it can be concluded that the composition of the medium influences the color and amount of the produced pigment, as proved by Shi *et al*. (2015).

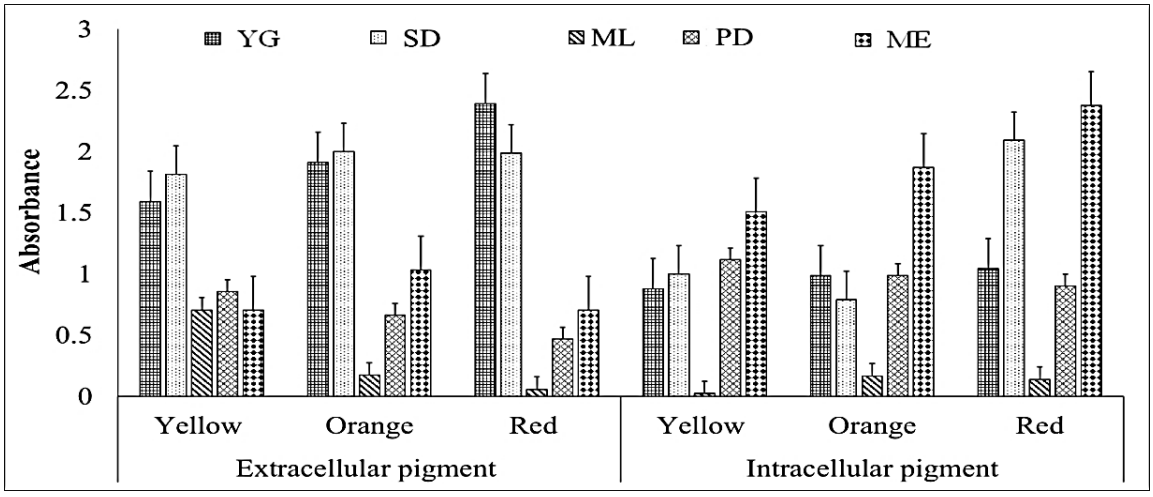


Fig. 7. Estimation of the produced pigments by *M. purpureus*.

Antimicrobial activity of Monascus pigments

M. purpureus showed antimicrobial activity against the G+ve *B. subtilis* and the G-ve *E. coli* but we observed that the influence was various with the different culture media. Table 2 showed that pigments extracted from YG medium filtrate were more effective as an antimicrobial agent against the tested bacteria. It was noticed that G+ve bacterium has been more sensitive to the action of *M. purpureus* pigment with an inhibition zone of 20.63 mm compared with the G-ve bacterium; the inhibition zone was 10.6 mm (Fig. 8).

Table 2. Antibacterial activity of different *M. purpureus* culture filtrates.

Tested bacteria	Zone of inhibition (mean ± SD, mm)				
	YGA	SDA	MEA	MLA	PDA
B. subtilis	20.63 ± 0.03	-ve	20.06 ± 0.06	-ve	20.46 ± 0.06
E. coli	10.6 ± 0	-ve	10.16 ± 0	-ve	10.5 ± 0

where -ve resembles no inhibition zone formation.

These results disagree with the results by Talapragada *et al*. (2017) that reported that the antimicrobial action of extract containing *M. purpureus* pigment had shown negative results against *E. coli* which may be attributed to the type of the media or tested strain.

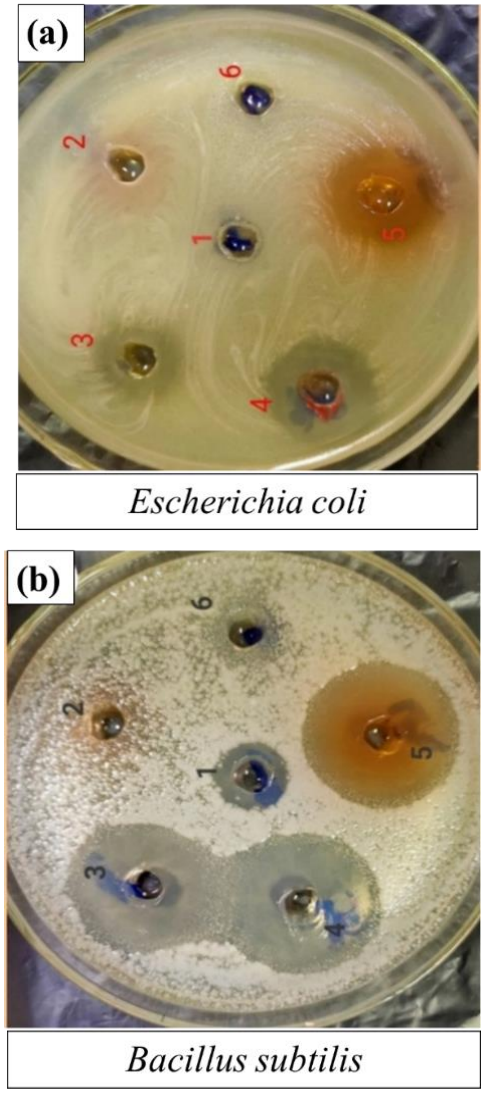


Fig. 8. Antimicrobial effect of *M. purpureus* pigments and AgNPs on *E. coli* (**a**) and *B. subtilis* (**b**) where (1): AgNPs, (2): SD, (3): Malt, (4): PD, (5): YG and (6): ML culture filtrate.

Abdel Ghany *et al*. (2015) reported that culture filtrate of *Monascus* strain had antibacterial action against *Bacillus* spp. Other research finds that antimicrobial activity is significantly determined by cell growth conditions and culture medium compositions (Narsing *et al*. 2017). The fungus *Monascus* can produce pigments, lovastatin (monacolin K), citrinin, dimerumic acid and c-aminobutyric acid, usually in the stationary growth phase. Some of these minor pigments might be intermediates or degradation products of the main pigments, and a possible relationship between monascusones A and B and the major yellow pigment, monascin, has been proposed (Jongrungruangchok *et al*. 2004). Lovastatin is a typical secondary metabolite that is produced in the stationary growth phase, and its production is subject to glucose repression. *M. pilosus* was induced to produce lovastatin in a liquid medium using a mixed substrate of maltose: glycerol (1 : 7) and peptone as the nitrogen source (Miyake *et al*. 2006). Orange *monascus* pigment has a weak antimicrobial activity, whereas the red pigment has little or no activity. A change in the oxygen part of orange pigment to a nitrogenous compound (amino acid) causes a color change to red (Saisadan *et al*. 2016). Lovastatin is a lactone that is readily hydrolyzed *in vivo* to the corresponding β-hydroxyacid and strong inhibitor of HMG-CoA reductase, a hepatic microsomal enzyme that catalyzes the conversion of HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) to mevalonate, an early rate-limiting step in cholesterol biosynthesis (Moghadasian 1999). Therapeutic properties of culture filtrate of *Monascus* spp. could be due to monascidin A, which is a secondary metabolite produced by *Monascus* spp. (Talapragada *et al*. 2017). Pigments colours of *M. purpureus* differed after extraction from the different culture media as shown in Fig. 9.

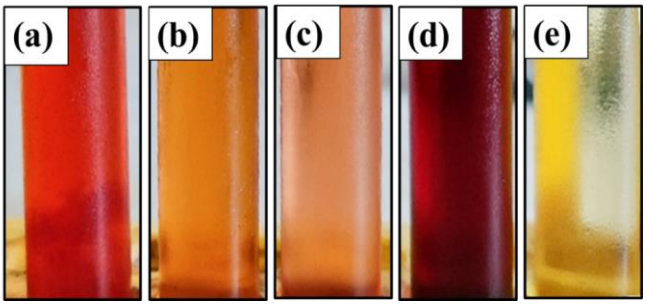


Fig. 9. Pigments extracts from *M. purpureus* were obtained after 7 days from static liquid cultivation at 30 ºC, in different culture media **(a)** SD, **(b)** ME, **(c)** PD, **(d)** YG and **(e)** ML broth media.

Antimicrobial activity of AgNPs

The results showed that there was visually yellowish-brown AgNPs as soon as the silver nitrate solution was added to the *M. purpureus* supernatant and then exposed to the sunlight (Fig. 10). In addition, *M. purpureus* filtrate showed no nitrate reductase activity which indicates that pigment is the main reducing power, and this result agrees with El-Batal *et al*. (2015) who reported that the high red pigment content of the *M. purpureus* culture filtrate could be responsible for the synthesis and stabilization of the AgNPs.

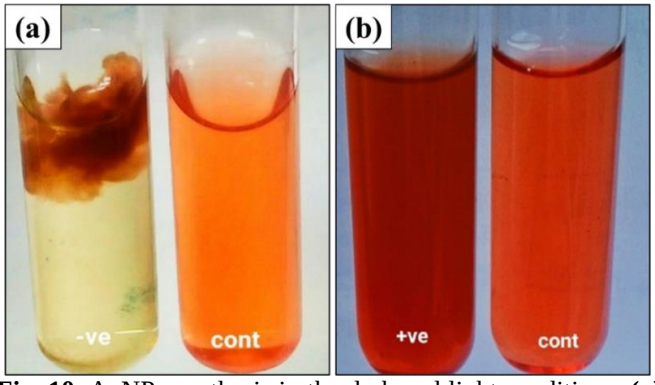
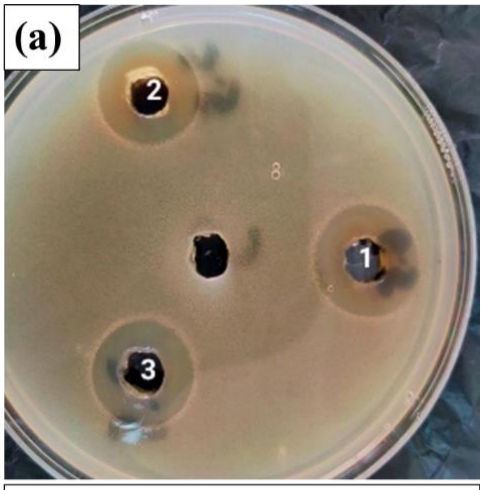


Fig. 10. AgNPs synthesis in the dark and light conditions. **(a)** *M. purpureus* showed no nitrate reductase activity, **(b)** showing AgNPs synthesis in the light whereas its pigments were reducing power.

Monascus red pigment-mediated AgNPs synthesis was evaluated for their antimicrobial activity against two pathogens, including one G+ve *(B. subtilis*) and one G-ve (*E. coli*) bacteria. The AgNPs inhibited the growth of the tested pathogens but showed higher antimicrobial activity towards *B. Subtilis* compared to *E. coli* (Fig. 11). *B. subtilis* was more susceptible to AgNPs showing the higher zone of inhibition 6 ± 0.1 mm at 10 µg.mL^{-1} AgNPs while *E. coli* with an inhibition zone of 1 mm and this was attributed to the effect of the nanoparticles. The interpretation of the reduction of the antibacterial activity by AgNPs instead of increasing with the pigments that *M. purpureus* pigments reducing power was decreased after nanoparticles formation (El-Baz *et al*. 2016). In this work, we also studied the effect of AgNPs that was synthesized at different pH values on bacterial growth and results showed that synthesized AgNPs at pH6.5 was more effective compared to the AgNPs that was synthesized at the other pH values and had higher distribution on the G+ve bacteria. Feng et al. (2000) reported different mechanisms of the antimicrobial action of nanomaterials such as penetrating the cell wall and plasma membrane ending with damaging DNA molecules. Others suggested that nanomaterials might interact with thiol groups in proteins, which induces the inactivation of microbial proteins. In the presented study, negatively charged *M. purpureus* EG crude metabolite compounds have an opposite charge with Gram-positive bacteria, in that way killing them more easily than Gram-negative bacteria due to the electrostatic attraction.

Koli *et al*. (2018) reported that the zone of inhibition increased by increasing the concentration of AgNPs and they determined the concentration of AgNPs which inhibit the 50 % growth of *E. coli* bacteria. The IC_{50} concentrations of AgNPs were observed to be 8.53 ± 0.6 µg.mL^{-1}.



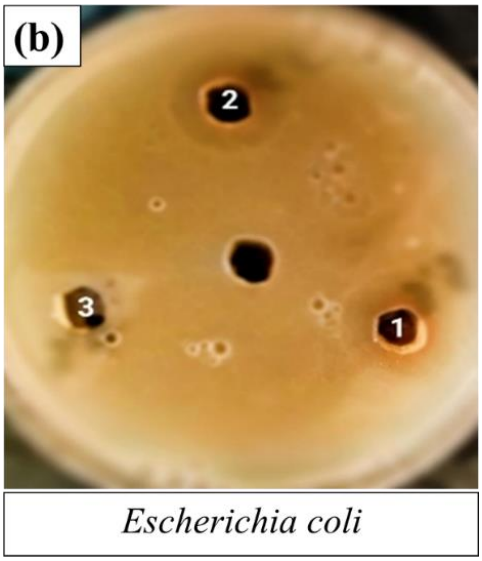


Fig. 11. Antibacterial effect of biosynthesized AgNPs at different pH values; (1) pH 6.5, (2) pH 9.5 and (3) pH 3.5.

The effect of pH values, on the AgNPs synthesis, could be illustrated in Fig. 12. The UV-VIS absorbance spectra of the different reaction peaks were 437, 453 and 447 nm for pH values 3.5, 6.5 and 9.5, respectively. The height of the UV-VIS spectrum and peak area, obtained from the reaction and carried out at pH 6.5, are considerably higher than those obtained at other pH values (Fig. 13) and indicates higher nanoparticle productivity. The obtained results, in the current study, are correlated with those obtained by Kim *et al*. (2002); Jaidev and Narasimha (2010); El-Zahed *et al*. (2019) and El-Zahed *et al*. (2021) studies that also showed the influence of AgNPs against G-ve and G+ve bacteria in addition, they added that there was a remarkable antifungal effect of AgNPs on *Candida albicans* and *S. cerevisiae*. This was in accordance with the results obtained by Sneha *et al*. (2010), who reported that low acidic and alkaline pH values decreased the AgNPs synthesis as silver tends to precipitate at alkaline pH, which could be due to the formation of the silver hydroxide. Similarly, El-Baz *et al.* (2016) recorded pH 6.5 as the best for silver nanoparticles biosynthesis.

Lately, the antimicrobial activity of negatively charged oxygen compounds (pigments) had been reported as the bioactive compounds in the biological control (Abdel Ghany *et al*. 2015). In the present study, the antimicrobial action of AgNPs has been associated with *M. purpureus* EG crude metabolite compounds chemical properties and their versatility such as deacetylation degree and molecular weight. This deacetylation action decreases the oxygen negative charge of pigments in addition to the positive charge of the silver ions that led to the decrease in the antimicrobial action of the bound AgNPs with *M. purpureus* EG crude metabolite compounds (Lin *et al*. 2019). Moreover, El-Baz et al. (2016) suggested that no nitrate reductase was detected by *M. purpureus*, whereas its pigments reducing power was decreased after nanoparticles formation indicating its role in the AgNPs biosynthesis. It was previously reported by Broder and Koehlerthat (1980) that the extracellular pigments were combined with the nitrogenous components of the medium. Also, the higher polarity of the red pigments was known to enhance their binding to water-soluble nitrogen-containing organic compounds, which has been proposed as the solubilization mechanism. On the other hand, recent research regarding the use of fungi has generally investigated potential redox systems using silver nitrate as the source of silver ions and *M. purpureus* extract having a 0.65 ± 0.03 reducing activity (equivalent cysteine, mM) (Vahabi *et al*. 2011). So, a further study of the synthesized AgNPs should be taken into account to study and improve the antimicrobial efficacy of AgNPs to have a negative surface charge throughout capping them with negatively charged proteins. The negative charge of the nanometal capping agent might increase the microbicidal effect of the *M. purpureus* EG crude metabolite compounds.

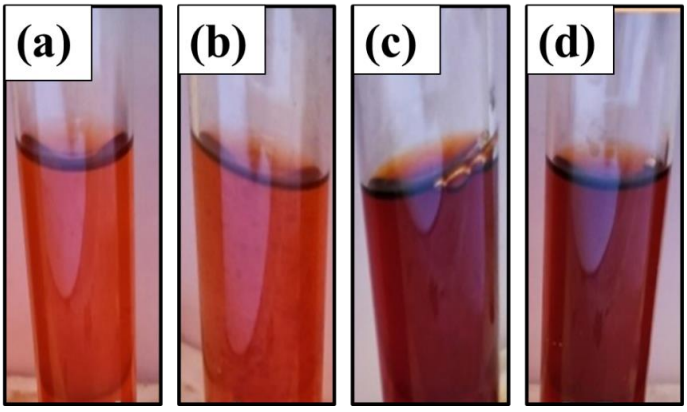


Fig. 12. Changing in the reaction colour before and after Ag^+ reduction into AgNPs by the action of *M. purpureus* culture filtrate at different PH values **(b)** 3.5, **(c)** 6.5 and **(d)** 9.5 in comparing with **(a)** negative control.

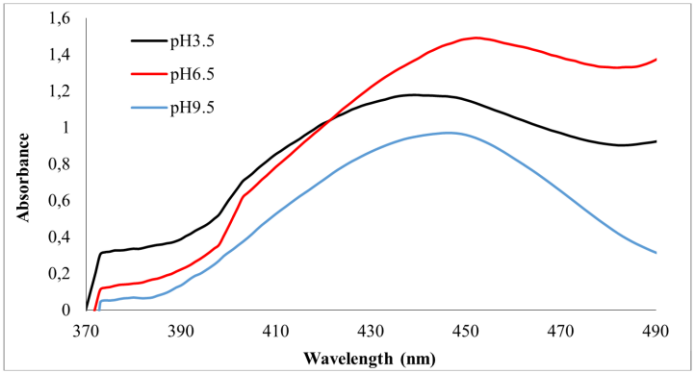


Fig. 13. UV-visible spectra of the produced AgNPs atdifferent pH values.

Conclusions

This study examined the effects of dark and light conditions on the growth and pigment yield of *M. purpureus* EG which revealed that the incubation in the total darkness was the most effective in induction of the pigment production and the colonies grew under the direct illumination resulted in less pigment production. This result postulates that there is an existence of photoreceptors responsive to dark and light in this fungus. The growth at 30 ºC gave the highest yield pigment. It can also be concluded that YG and malt extract broth media were the most favourable to produce extracellular and intracellular pigment of *M. purpureus*, respectively. These conditions might be beneficial in the production of *Monascus* pigments for use in the food industry. *M. purpureus* showed antimicrobial activity against Gram-positive and negative bacterial strains, so its secondary metabolites appeared to have a great potential in medicinal uses. Finally, the synthesis of biogenic AgNPs was considered easy, rapid, safe, and did not need any additional toxic reducing agent as extracellular red pigments are used as a reducing and natural capping agent. Moreover, the AgNPs had shown antibacterial activity against G+ve and G-ve bacterial strains which might be used in the biomedical application and environmental field.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Abdel Ghany T, Shater A, Negm M, Al Abboud M, Elhussieny N (2015) Efficacy of botanical fungicides against *Curvularia lunata* at molecular levels. J. Plant Pathol. Microb. 6: 289-296.

Arunachalam C, Narmadhapriya D (2011) *Monascus* fermented rice and its beneficial aspects: a new review. AJPCR 4: 29-31.

Babitha S, Carvahlo JC, Soccol CR, Pandey A (2008) Effect of light on growth, pigment production and culture morphology of *Monascus purpureus* in solid-state fermentation. World. J. Microb. Biot. 24: 2671-2675.

Ballario P, Macino G (1997) White collar proteins: PASsing the light signal in *Neurospora crassa*. Trends Microbiol. 5: 458-462.

Beever RE, Bollard EG (1970) The nature of the stimulation of fungal growth by potato extract. J. Gen. Microbiol. 60: 273-279.

Broder CU, Koehler PE (1980) Pigments produced by *Monascus purpureus* with regard to quality and quantity. J. Food Sci. 45: 567-569.

Carvalho JCD, Oishi BO, Pandey A, Soccol CR (2005) Biopigments from *Monascus*: strains selection, citrinin production and color stability. Braz. Arch. Biol. Technol. 48: 885-894.

Chen MT, Tseng YY (1989) Efficacy of antimicrobial substances from Monoascus metabolites on preservation of meat. In 35th International Congress of Meat Science and Technology Proceeding, vol. 2, pp. 483-485, Copenhagen, Denmark.

Choe D, Song SM, Shin CS, Johnston TV, Ahn HJ, Kim D, Ku S (2020) Production and Characterization of Anti-Inflammatory *Monascus* Pigment Derivatives. Foods 9: 858.

El-Batal AI, El-Baz AF, Abomosalam FM, Tayel AA (2015) Improvement of silver nanoparticles synthesis by *Monascus purpureus* using Gamma irradiation. RJAB 1: 80-100.

El-Baz AF, El-Batal AI, Abomosalam FM, Tayel AA, Shetaia YM, Yang S-T (2016) Extracellular biosynthesis of anti-*Candida* silver nanoparticles using *Monascus purpureus*. J. Basic Microbiol. 56: 531-540.

El-Sayed ESR, Abdelhakim HK and Zakaria Z (2020) Extracellular biosynthesis of cobalt ferrite nanoparticles by *Monascus purpureus* and their antioxidant, anticancer and antimicrobial activities: yield enhancement by gamma irradiation. Mater. Sci. Eng. C 107: 110318.

El-Zahed MM, Baka ZA, Abou-Dobara MI, El-Sayed AKA (2019) Synthesis, characterization and antimicrobial activity of chitosan/Ag nanocomposite using *Escherichia coli* D8. SJDFS 9: 1-6.

El-Zahed MM, El-Dein MMN, Baka ZAM, Abou-Dobara MI, El-Sayed AKA (2021) Extracellular biosynthesis, optimization, characterization and antimicrobial potential of *Escherichia coli* D8 silver nanoparticles. J. Microbiol. Biotechnol. Food Sci. 10: 648-656.

Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO (2000) A mechanistic study of the antibacterial effect of silver

ions on *Escherichia coli* and *Staphylococcus aureus*. J. Biomed. Mater. Res. 52: 662-668.

Guo L, Breakspear A, Zhao G, Gao L, Kistler HC, Xu J, Ma L (2016) Conservation and divergence of the cyclic adenosine monophosphate-protein kinase A (cAMP–PKA) pathway in two plant-pathogenic fungi: *Fusarium graminearum* and *F. verticillioides*. Mol. Plant Pathol. 17: 196-209.

Haque MA, Kachrimanidou V, Koutinas A, Lin CSK (2016) Valorization of bakery waste for biocolorant and enzyme production by *Monascus purpureus*. J. Biotechnol. 231: 55-64.

Huang ZR, Zhou WB, Yang XL, Tong AJ, Hong JL, Guo WL, Li TT, Jia RB, Pan YY, Lin J, Lv XC, Liu B (2018) The regulation mechanisms of soluble starch and glycerol for production of azaphilone pigments in *Monascus purpureus* FAFU618 as revealed by comparative proteomic and transcriptional analyses. Food Res. Int. 106: 626-635.

Huawei Z, Chengtao W, Jie Q, Bingjing Z, Bing Z, Chuangyun (2019) D Determining a suitable carbon source for the production of intracellular pigments from Monascus purpureus HBSD 08. Pigm Resin Technol 48: 547-554.

Jacobs MB, Gerstein MJ (1960) Handbook of Microbiology. New York: Van Nostrand Co., 322 p.

Jaidev LR, Narasimha G (2010) Fungal mediated biosynthesis of silver nanoparticles, characterization and antimicrobial activity. Colloids Surf B 81: 430-433.

Jongrungruangchok S, Kittakoop P, Yongsmith B, Bavovada R, Tanasupawat S, Lartpornmatulee N, Thebtaranonth Y (2004) Azaphilone pigments from a yellow mutant of the fungus *Monascus kaoliang*. Phytochemistry 65: 2569-2575.

Kim HJ, Kim JH, Oh HJ and Shin CS (2002) Morphology control of *Monascus* cells and scale-up of pigment fermentation. Process Biochem. 38: 649-655.

Koli SH, Mohite BV, Suryawanshi RK, Borase HP, Patil SV (2018) Extracellular red *Monascus* pigment-mediated rapid one-step synthesis of silver nanoparticles and its application in biomedical and environment. Bioproc. Biosystems Eng. 41: 715-727.

Lee BK, Park NH, Piao HY, Chang WJ (2001) Production of red pigments by *Monascus purpureus*. Submerged Culture Biotechnol. Bioprocess Eng. 6: 341-346.

Lin CS, Hur HF, Lin CC (2019) Antioxidant properties and antibacterial activity of fermented *Monascus purpureus* extracts. MOJFPT 7: 49-54.

Liu J, Du Y, Ma H, Pei X, Li M (2020) Enhancement of *Monascus* yellow pigments production by activating the cAMP signalling pathway in *Monascus purpureus* HJ11. Microb. Cell Fact. 19: 1-11.

Malik K, Tokkas J, Goyal S (2012) Microbial pigments: a review. Int. J. Microb. Resour. Technol. 1: 361-365.

Miyake T, Mori A, Kii T, Okuno T, Usui Y, Sato F and Kariyama M. 2005. Light effects on cell development and secondary metabolism in *Monascus*. J. Ind. Microbiol. Biotechnol. 32: 103-108.

Miyake T, Uchitomi K, Zhang M-Y, Kono I, Nozaki N, Sammoto H, Inagaki K (2006) Effects of the principal nutrients on lovastatin production by *Monascus pilosus*. Biosci. Biotechnol. Biochem. 70: 1154-1159.

Moghadasian MH (1999) Clinical pharmacology of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Life Sci. 65: 1329-1337.

Narsing Rao MP, Xiao M, Li WJ (2017) Fungal and bacterial pigments: secondary metabolites with wide applications. Front. Microbiol. 8: 1113.

Park CD, Jung HJ, Lee HW, Kim HS, Yu TS (2005) Antioxidant activity of *Monascus* pigment of *Monascus purpureus* P-57 mutant. Korean J. Microbiol. 41: 135-139.

Patrovsky M, Sinovska K, Branska B, Patakova P (2019) Effect of initial pH, different nitrogen sources, and cultivation time on the production of yellow or orange *Monascus purpureus* pigments and the mycotoxin citrinin. Food Sci. Nutr. 7: 3494-3500.

Pengnoi P, Mahawan R, Khanongnuch C, Lumyong S (2017) Antioxidant properties and production of monacolin k, citrinin, and red pigments during solid state fermentation of purple rice (*Oryzae sativa*) varieties by *Monascus purpureus*. Czech J. Food Sci. 35: 32-39.

Pisareva EI, Kujumdzieva AV (2010) Influence of carbon and nitrogen sources on growth and pigment production by *Monascus pilosus* C1 strain. Biotechnol. Biotechnol. Equip. 24: 501-506.

Saisadan D, Manimaran P, Meenapriya PK (2016) *In vitro* comparative evaluation of mechanical properties of temporary restorative materials used in fixed partial denture. J. Pharm. Bioaplied. Sci. 8: S105-S109.

Seenivasan A, Sankar P, Gummadi SN, Panda T, Venkateswarlu Ch (2020) Metabolic pathway analysis and dynamic macroscopic model development for lovastatin production by *Monascus purpureus* using metabolic footprinting concept. Biochem. Eng. J. 154: 107437.

Sharmila G, Nidhi B, Muthukumaran C (2013) Sequential statistical optimization of red pigment production by *Monascus purpureus* (MTCC 369) using potato powder. Ind. Crop. Prod. 44: 158-164.

Shi K, Song D, Chen G, Pistolozzi M, Wu Z, Quan L (2015) Controlling composition and color characteristics of *Monascus* pigments by pH and nitrogen sources in submerged fermentation. J. Biosci. Bioeng. 120: 145-154.

Sneha K, Sathishkumar M, Mao J, Kwak IS, Yun YS (2010) *Corynebacteriumglutamicum*-mediated crystallization of silver ions through sorption and reduction processes. Chem. Eng. J. 162: 989-996.

Takeshita R, Saigusa N, Teramoto Y (2016) Production and antioxidant activity of alcoholic beverages made from various cereal grains using *Monascus purpureus* NBRC 5965. J. Inst. Brew. 122: 350-354.

Talapragada P, Dikshit R, Phocas M, MR M, Samprathi S (2017) Effect of amino acids on pigments, citrinin, and lovastatin production by *Monascus purpureus* under static conditions. Biologija 63: 160-168.

Thom C, Church M (1926) The Aspergilli. Baltimore: Williams & Wilkins. 163 p.

Vahabi K, Mansoori GA, Karimi S (2011) Biosynthesis of silver nanoparticles by fungus *Trichodermareesei*. Insciences J. 1: 65-79.

Waksman SA (1967) The actinomycetes."A summary of current knowledge". Ronald press, New York. 280 p.

Yang X, Dong Y, Liu G, Zhang C, Cao Y and Wang C (2019) Effects of nonionic surfactants on pigment excretion and cell morphology in extractive fermentation of *Monascus* sp. NJ1. J. Sci. Food Agric. 99: 1233-1239.

Zhang C, Zhang H, Zhu Q, Hao S, Chai S, Li Y, Wang C (2020) Overexpression of global regulator LaeA increases secondary metabolite production in *Monascus purpureus*. Appl. Microbiol. Biotechnol. 104: 3049-3060.