ANTIMICROBIAL PIGMENT FROM FUSARIUM GRAMINEARUM: OPTIMIZING CONDITIONS AND UTILIZING AGRO-INDUSTRIAL RESIDUES

Tugce DAG², Gulcan SAHAL*¹, Isil Seyis BILKAY¹

Address(es): Dr. Gulcan Sahal, ¹Hacettepe University, Faculty of Sciences, Department of Biology (Biotechnology Section), 06800 Ankara, Turkey.
²Corresponding author: gulcanozbakir@gmail.com

INTRODUCTION

Pigments are essential compounds that are widely used in industry, pharmacology, and medicine (Delgado et al., 2000; Malik et al., 2012) and are obtained both naturally and synthetically. Toxicity problems caused by synthetic pigments; carcinogenic properties of precursors used in the production of them and industrial byproducts of them on humans and the environment pose a danger in synthetic pigment production and use (Boonyaprapai et al., 2008). By this means, natural dyes and pigments in foodstuff, cosmetic and pharmaceutical manufacturing processes are regarded as important alternatives to potentially harmful synthetic dyes (Velmurugan et al., 2010) and are becoming increasingly popular day by day (Boonyaprapai et al., 2008; Velmurugan et al., 2010). Apart from this, since agro-industrial residues such as molasses and whey can also be used by various microorganisms as low-cost growth media, the production of pigments from microorganisms also provides conversion of industrial wastes into valuable bioproducts through biological processes (Mehri et al., 2021). On the other hand, as a result of growing easily and quickly in a low-cost culture media and not being affected by the changes in climatic conditions, the production of pigments from microorganisms is an efficient and economical process compared to the production of pigments synthetically (Boonyaprapai et al., 2008; Bouhrir et al., 2020; Velmurugan et al., 2010; Waghela and Khan, 2018). Moreover, the efficiency of pigment production can be easily improved by optimization of cultural parameters, mutation, and genetic engineering techniques when microbial sources are used (Mapari et al., 2005). Microbial pigments are also reported to avoid food spoilage by displaying antimicrobial effects on various food pathogens (Konuray and Erginkaya, 2015). Considering the reasons for natural pigment usage in the food industry as: i) enhancing the present color of the food, ii) coloring the uncolored food, and iii) supplementing food with nutrients (Konuray and Erginkaya, 2015): antimicrobial natural pigments are preferable also to improve the shelf life of a particular foodstuff. Fusarium species are cosmopolitan, soilborne, and filamentous fungi known to cause a huge range of diseases on an extraordinary range of host plants (Singha et al., 2016). Despite causing vascular wilt or root rot diseases in agricultural and ornamental crops throughout the world; many species belonging to Fusarium sp. are reported to produce various bioactive pigments widely used in pharmaceuticals, cosmetics, and the food industry (Malini, 2018). The objective of this study was to investigate the antimicrobial effect of the pigment produced by the highest pigment-producing Fusarium sp. strain, determine the optimal growth conditions for maximum pigment production, and assess the feasibility of conversion of agro-industrial wastes such as molasses and whey as low-cost growth media for pigment production by Fusarium graminearum. This study promotes the development of natural and antimicrobial pigment production, while also utilizing agro-industrial wastes as sustainable resources.

MATERIAL AND METHODS

Fusarium species

In this study, Fusarium equiseti, Fusarium graminearum, and Fusarium poae strains which were obtained from the Culture Collection of our Laboratory in the Biology Department (Biotechnology Division) of Hacettepe University were used. These cultures were grown in Potato Dextrose Agar (PDA) (Merck) media for 7 days at 30 ºC and stored at +4 ºC for use in further experiments.

Inoculum preparation and growth conditions

For inoculum preparation, the fungal strains were first grown on a PDA medium for 7 days at 30 ºC. Following incubation, 5 mm² plugs from the outer zone of the fungal colony were taken out with a sterile inoculation loop and transferred into a 10 mL sterile saline solution to obtain inoculums as fungal suspensions. 10 mL fungal suspensions were grown in a 250 mL flask containing 100 mL of Potato Dextrose Broth (PDB), Sabouraud Dextrose Broth (SDB), and Malt Extract Broth (MEB) (Merck), with an initial pH level of 5.5 for 7 days at 30 ºC and 150 rpm rotation speed (Pradeep and Pradeep, 2013).

Dry cell weight and pigment quantification

Incubated fungal cultures were first filtered with a filter paper and the mycelia biomass yield was dried at 30 ºC for 48 h. Following this, filtered supernatants were centrifugated at 3220g for 10 min at 10 ºC (Eppendorf 5810R, with an Eppendorf Swing-bucket rotor A-4-62, Hamburg, Germany). After centrifugation, supernatants were evaluated in terms of pigment production with the method described by Pradeep et al., (2013). Briefly, quantification of the fungal pigments was done by measuring the absorbance of the broth after centrifugation at 500 nm using a spectrophotometer (UV–Visible Spectrophotometer; Shimadzu UV-1700, Kyoto, Japan). This study evaluated the absorbance of PDB, SDB, and MEB
without any growth as negative control and set as zero in terms of pigment production.

Antimicrobial effect of the extracted pigment

In this part of the study, E. coli ATCC 25922, Klebsiella pneumoniae ATCC 13883, Pseudomonas aeruginosa ATCC 27853, Salmonella paratyphi ATCC 9150, Staphylococcus aureus ATCC 25923 strains which were obtained from the Culture Collection of our laboratory in the Biology Department (Biotechnology) of Hacettepe University and Bacillus cereus isolate (Reference Strain: Bacillus cereus ATCC 14579) (Ozyurek and Bilikay, 2017) were used. The antimicrobial effect of the extracted F. graminearum pigment against these microbial strains was determined. These microbial strains were first grown in Nutrient Agar at 37 °C for 24 hours and stored at +4 °C for use in further experiments.

Pigment extraction

The pigment produced by F. graminearum was extracted by the solvent extraction method, described by Velmurugan et al. (2009). Briefly, 10 mL F. graminearum suspensions were grown in 100 mL of MEB with pH 5.5 and incubated at 30 °C, 150 rpm rotation speed for 7 days. Following incubation, F. graminearum culture was filtered with a filter paper and the supernatant was centrifugated at 3220g for 10 min at 10 °C (Eppendorf 5810R, with an Eppendorf Swing rotor A-4-62, Hamburg, Germany). After centrifugation, 10 mL of the centrifugated supernatant was mixed with 20 mL ethanol solution (90%) inside the tubes. Following this, these tubes were kept inside the shaking incubator at 200 rpm rotation speed for 1 hour. Then, the tubes were kept for 15 min at 25 °C. Then, the pigment-ethanol mixture was filtered with Whatman no.1 filter paper and the filtered mixture including fungal pigment was dried at 30 °C for 48 hours. The dry pigment (20 mg) was dissolved in 1 mL sterile distilled water and kept to be used for the antimicrobial tests.

Antimicrobial test

The antimicrobial effect of the extracted pigment against the selected microorganisms was determined by Disk Diffusion and Agar Well Diffusion Methods (Bilen Ozyurek et al., 2017; Hudzicki, 2012). Briefly, single colonies of different bacterial strains were inoculated into 10 mL BHI broth (Lab M Ltd, Lancashire, UK) and incubated for 24 hours at 37 °C. After incubation, cells were harvested by centrifugation for 10 min at 10 °C (Eppendorf Centrifuge 5417C, Germany) washed 3 times with sterile saline solution, and were adjusted to 0.5 McFarland standard turbidity inside the sterile saline solution. 100 µL of a microbial suspension corresponding to a 0.5 McFarland standard turbidity was added to the Mueller-Hinton Agar medium and spread over the entire agar surface. After waiting for 15 min, pits with 1 cm diameter were prepared under sterile conditions on the agar plate. 20 µL of the extracted pigment, which was sterilized for an hour under UV light, was added to the previously prepared pits. All the plates were incubated for 24 h at 37°C and the diameters of zones of inhibitions (ZOI) were measured using a ruler. For the determination of the antimicrobial effect of the extracted pigment, 20 µL sterile distilled water was used as a negative control; whereas Vancomycin (30 µg/disk) disk was used as a positive control.

Determination of optimum growth parameters for pigment production by F. graminearum

Effect of initial pH, temperature, different incubation conditions and periods were assessed to determine optimum growth parameters for pigment production by F. graminearum (Boonyapranai et al., 2008).

Effect of initial pH

10 mL F. graminearum suspensions were grown in a 250 mL flask containing 100 mL of MEB with different pH levels covering the range from pH: 5 to 10 and incubated for 7 days at 150 rpm rotation speed, keeping the temperature constant at 30 °C for each group. Following incubation with different pH levels, dry cell weight and pigment quantification were assessed as previously described.

Effect of Temperature

10 mL F. graminearum suspensions were grown in a 250 mL flask containing 100 mL of MEB and incubated at three different temperatures as 25 °C, 30 °C, and 37 °C for 7 days at 150 rpm rotation speed, keeping pH constant at 5.5 for each group. Following incubation at different temperatures, dry cell weight and pigment quantification were assessed as previously described.

Effect of incubation under static/shaking conditions

10 mL F. graminearum suspensions were grown in a 250 mL flask containing 100 mL of MEB with a constant pH of 5.5 and incubated for 7 days, keeping the temperature also constant at 30 °C for each group under static and shaking (at 150 rpm rotation speed) conditions. Following incubation under static and shaking conditions, dry cell weight and pigment quantification were assessed as previously described.

Effect of incubation under light/dark conditions

10 mL F. graminearum suspensions were grown in a 250 mL flask containing 100 mL of MEB with a constant pH of 5.5 and incubated for 7 days, keeping the temperature also constant at 30 °C for each group under a light source (Cool White; Philips TS, Master TL-D, TS 51, Watt 840) and dark conditions at 150 rpm rotation speed. Following incubation under a light source and dark conditions, dry cell weight and pigment quantification were assessed as previously described.

Effect of incubation period in days

10 mL F. graminearum suspensions were grown in a 250 mL flask containing 100 mL of MEB and incubated for different incubation periods covering the range from 2 days to 10 days at 150 rpm rotation speed, keeping the temperature constant at 30 °C and the initial pH as 5.5 for each group. Following different incubation periods, pigment quantification was established as previously described. In this part of the study, pigment quantification was assessed every 24 hours from the same growing F. graminearum culture. Therefore, F. graminearum culture continued growing every day and dry cell weight was unable to be calculated for each day of incubation.

Pigment production from agro-industrial residues

Pigment production from agro-industrial residues was carried out using a modified version of the method described by Lopes et al., 2013. In this part of the study, molasses, Turkish Cheddar Cheese Whey (TCCW), and Turkish Feta Cheese Whey (TFCW) as agro-industrial residues were used for pigment production. Molasses was obtained from one of the sugar factories in Ankara, Turkey; whereas TCCW and TFCW were obtained from One of the farm factories in Ankara, Turkey. Both deproteinized and non-deproteinized forms of TCCW and TFCW and their 10^-2 dilutions and 10^-4 times diluted molasses were tested in this study. To prepare deproteinized whey, TCCW and TFCW were boiled for 10 min. Afterward, they were centrifugated at 3220g for 10 min at 10°C (Eppendorf 5810R, with an Eppendorf Swing-bucket rotor A-4-62, Hamburg, Germany) and filtered through Whatman no.1 filter paper. The filtered supernatant was sterilized under autoclave at 110 °C, 1.5 atm for 15 min. For inoculum preparation, the fungal strains were first grown on a PDA medium for 7 days at 30 °C. Following incubation, 1 mm² plug from the outer zone of the fungai colony was taken out with a sterile inoculation loop and transferred into a 250 mL flask containing 100 mL MEB with an initial pH level of 5.5, and the flask was incubated for 7 days at 30 °C and 150 rpm rotation speed. For pigment production from agro-industrial residues, 5 mL F. graminearum, grown in 100 mL MEB was transferred into: i) 100 mL 10^-2 diluted molasses (sterilized under autoclave at 110 °C, 1.5 atm for 15 min.), ii) 100 mL 10^-2 diluted TFCW (sterilized under autoclave at 110 °C, 1.5 atm for 15 min.), iii) 100 mL 10^-2 diluted TFCW (sterilized under autoclave at 110 °C, 1.5 atm for 15 min.), iv) 100 mL 10^-2 diluted TFCW (sterilized under autoclave at 110 °C, 1.5 atm for 15 min.), v) 100 mL 10^-2 diluted TCCW (sterilized under autoclave at 110 °C, 1.5 atm for 15 min.), vi) 100 mL 10^-2 diluted TCCW (sterilized under autoclave at 110 °C, 1.5 atm for 15 min.), vii) 100 mL 10^-2 diluted TCCW (sterilized under autoclave at 110 °C, 1.5 atm for 15 min.), viii) 100 mL 10^-2 diluted TCCW (sterilized under autoclave at 110 °C, 1.5 atm for 15 min.), ix) 100 mL 10^-2 diluted TCCW (sterilized under autoclave at 110 °C, 1.5 atm for 15 min.), x) 100 mL 10^-2 diluted TCCW (sterilized under autoclave at 110 °C, 1.5 atm for 15 min.), y) 100 mL 10^-2 diluted TCCW (sterilized under autoclave at 110 °C, 1.5 atm for 15 min.), z) 100 mL 10^-2 diluted TCCW (sterilized under autoclave at 110 °C, 1.5 atm for 15 min.). Incubation was applied for 7 days at 30 °C and 150 rpm rotation speed with an initial pH level of 8. Pigment quantification was assessed for F. graminearum grown in molasses by the method, described and the amounts of pigments, produced in whey were observed visually.

Statistical Analysis

The Shapiro–Wilk test was conducted to analyze the normality of the data. In case the results were found as normally distributed, a t-test was used for evaluation of our results. P<0.05 was considered statistically significant. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 23 Software (IBM Corp, New York, USA).

RESULTS AND DISCUSSION

Colors have been widely used in various industries such as food, textile, cosmetics, and pharmaceuticals for many years (Tuli et al., 2015). However, hyper allergenic, carcinogenic, and toxicological problems caused by most synthetic colorants have made it necessary to replace synthetic pigments with natural pigments and use natural colors obtained from pigment-producing organisms including plants, animals, and microorganisms as colorants (Reyes et al., 1996; Tuli et al., 2015). Among all these organisms, microorganisms are generally considered as main sources of natural pigment production due to their availability, stability, and cost efficiency (Tuli et al., 2015).

Growth and pigment production of Fusarium equiseti, Fusarium graminearum, and Fusarium poae species in different media

In the first part of this study, we tested growth and pigment production by Fusarium graminearum, Fusarium poae, and Fusarium equiseti species, in three different media as Malt Extract Broth (MEB), Potato Dextrose Broth (PDB), and
Sabouraud Dextrose Broth (SDB). Pigment production by all *Fusarium* species were found to be normally distributed and therefore t-test was applied revealing that pigment production statistically differs from each other. According to our results, the highest amount of pigment production by all *Fusarium* species was observed in MEB (Fig. 1). Since significantly more pigment production by *F. graminearum* in MEB media (p<0.001) was observed, both *F. graminearum* and MEB media were selected to be used for further experiments. Our finding that shows significantly higher pigmentation of all the three tested *Fusarium* species in MEB (p<0.001), was in line with one of the studies, reporting lighter pigmentations in the media without organic nitrogen sources such as peptone, yeast extract, and malt extract (Pradeep *et al.*, 2015).

**Figure 1** Pigment production and growth by *Fusarium graminearum*, *Fusarium poae*, and *Fusarium equiseti* strains in A) Malt Extract Broth, B) Potato Dextrose Broth, C) Sabouraud Dextrose Broth media. Error bars indicate standard deviations over triplicate experiments with separately grown fungal cells

**Antimicrobial effect of the extracted *F. graminearum* pigment**

*F. graminearum* colonizes on wheat plant surfaces with specialized unbranched hyphae called runner hyphae (RH) and is known as one of the most destructive plant pathogens worldwide, causing fusarium head blight (FHB) on cereals (Mentges *et al.*, 2020). Despite its destructive effect and being responsible for losses of over $2.7$ billion in the United States between 1998 and 2000; *F. graminearum* is one of the most important fungal species by producing pigments such as aurofusarin, rubrofusarin, and culmorin (Cambaza, 2018). The reddish pigment deposition in the cell walls (Malz *et al.*, 2005) in one of the previous studies. However recently, rubrofusarin synthesized by *F. graminearum* has been reported to be a red naphthoquinone pigment having antimicrobial, antiallergic, and phytotoxic properties (Poorniammal *et al.*, 2021). In this part of the study, the antimicrobial effect of the extracted *F. graminearum* pigment against 5 different bacterial species (*Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) were tested. According to our results, extracted *F. graminearum* pigment exhibited an antimicrobial effect against *B. cereus* and *S. aureus* strains (Table 1 and Fig. 2).

**Table 1** Antimicrobial effect of the extracted *F. graminearum* pigment against *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, and *Staphylococcus aureus* strains by disc diffusion method.

<table>
<thead>
<tr>
<th>Tested Microorganisms</th>
<th><em>B. cereus</em></th>
<th><em>E. coli</em></th>
<th><em>K. pneumoniae</em></th>
<th><em>P. aeruginosa</em></th>
<th><em>S. paratyphi</em></th>
<th><em>S. aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile Distilled Water (20 µL)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Pigment (20 µL)</td>
<td>10.2</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>14.9</td>
</tr>
<tr>
<td>Vancomycin (30 µg)</td>
<td>15.5</td>
<td>15.4</td>
<td>15.6</td>
<td>15.7</td>
<td>15.5</td>
<td>16.1</td>
</tr>
</tbody>
</table>
Among these *F. graminearum* pigment sensitive bacterial strains; *B. cereus* is known to be responsible for Food-Borne Diseases (FBD) such as diarrheal and an emetic illness that is caused by the ingestion of foods contaminated with *B. cereus* (Griffiths et al., 2017). Apart from this, *S. aureus* is also reported as one of the significant causes of FBD, causing an estimated 241,000 illnesses per year in the United States (Kadariya et al., 2014). By this means, according to our findings, the reddish pigment of *F. graminearum* that has been investigated in this study can be one of the most useful bio-colorants in the food industry not only because it is derived from a microbial source but also because it inhibits some food contaminants such as *B. cereus* and *S. aureus* that have been responsible for various FBD for years. In this respect, optimum incubation conditions to obtain the highest amount of pigment production by *F. graminearum* were aimed to be determined for the next part of this study.

**Determination of optimum growth parameters for pigment production by *F. graminearum***

In this part of this study, the growth rate of *F. graminearum* was nearly the same when the pH values are adjusted to pH 5-8 (Fig. 3A). However, higher pigment production by *F. graminearum* was observed between pH levels of 6 and 8 (Fig. 3A). Among all the pH levels, the highest pigment production by *F. graminearum* was observed under the pH level of 8 by causing significantly higher pigment production compared pH levels of 5, 6 and 7 (<0.001) (Fig. 3A). Following the increase in pH level (pH > 8) both the growth rate of and the pigment production by *F. graminearum* decreased (Fig. 3A). To determine the optimum temperature level for the reddish pigment production by *F. graminearum*; temperature levels of 25, 30 and 37 °C were tested. Reddish pigment production and growth were observed at 25 °C and 30 °C (Fig. 3B). However, pigment production by *F. graminearum* at 30 °C was significantly higher than the pigment production at 25 °C and 30 °C (<0.001) (Fig. 3B). At 37 °C, both the growth rate of and the pigment production by *F. graminearum* decreased (Fig. 3B). When the pigment production by *F. graminearum* is tested under static and shaking incubation conditions, reddish pigment production and growth was observed only in shaking incubation conditions (Fig. 3C). Apart from this, although there was growth under light and dark incubation conditions, reddish pigment production by *F. graminearum* was observed only in the light provided incubation conditions (Fig. 3D). Fig. 3E shows the effect of incubation periods in days on the reddish pigment production by *F. graminearum*. According to our results, reddish pigment production increasingly continued until the 9th day of incubation and a significant decrease was observed afterward (<0.05). Therefore, 9 days of incubation was determined as the optimum incubation day for the reddish pigment production by *F. graminearum*. To sum up, optimum pigment production by *F. graminearum* was observed under light-provided conditions, with an initial pH level of 8, at 150 rpm shaking speed, 30 °C temperature and for 9 days of incubation (Fig. 3).

**Figure 2** Images of antimicrobial effect of the extracted *F. graminearum* pigment against *B. cereus* and *S. aureus* strains (Red arrows present zone of inhibitions)

**Figure 3** Pigment production and growth by *Fusarium graminearum* (A) in different pH levels, B) in different temperatures (°C), C) in static and shaking incubation conditions, D) under light and dark incubation conditions, E) pigment production by *Fusarium graminearum* under different incubation periods (in days). Error bars indicate standard deviations over triplicate experiments with separately grown fungal cells. (Detail information on growth conditions that are kept constant is given).
Similar to our findings, in the studies carried out on pigment production by *Fusarium* sp., the highest mycelial growth and pigment production have been reported to be seen at 28 °C temperature for 8-10 days of incubation under shaking conditions (Malini, 2019; Pradeep et al., 2013). However, pH levels of 5 and 5.5 have been reported as the optimum levels for the highest growth and pigment production by *F. graminearum* (Malini, 2019; Pradeep et al., 2013). Therefore, the pH level has been kept constant at 5.5, during the determination of the optimum growth parameters for pigment production by *F. graminearum*. However, different from the other studies carried out on pigment production by *Fusarium* sp.; the highest mycelial growth and pigment production was seen at a pH level of 8 in our study (p<0.001).

**Pigment production by *F. graminearum* from agro-industrial residues**

Agro-industrial residues are known as many different wastes from the food and agriculture industry including multiple plant-based materials, such as straws, stems, stalks, leaves, husks, shells, peels, lint, seeds, pulps, stubbles, bagasse, spent coffee grounds, brewer’s spent grains, and some animal byproducts, including feathers and whey which are also known to be rich in nutrient components, such as carbohydrates, proteins, fibers, minerals, and vitamins (Lopes and Ligabue-Braum, 2021). Nowadays, researchers have focused on various biotechnological approaches that aim to convert food waste into valuable bioproducts through various biological processes (Mehri et al., 2021). In accordance with this aim, the effect of molasses and whey on the reddish pigment production by *F. graminearum* was tested in the last part of our study. According to our findings, 10^2 times diluted molasses was suitable for reddish pigment production by *F. graminearum* (Fig. 4). Apart from this, although the growth of *F. graminearum* was significantly lower in 10^2 diluted versions of TFCW and TCCW (p<0.001), more reddish pigment production by *F. graminearum* was visually observed in 10^2 diluted versions of TFCW and TCCW (Fig. 5). Additionally, an increase in reddish color was also visually observed after deproteinization of undiluted TFCW and TCCW (Fig. 5).

**Figure 4** Pigment production and growth by *F. graminearum* after incubation in 10^2 diluted Molasses. Incubation was provided for 7 days under light conditions at 30 °C, 150 rpm rotation speed with an initial pH level of 8. Error bars indicate standard deviations over triplicate experiments with separately grown fungal cells.

**Figure 5** Growth of *F. graminearum* after incubation in different TFCW and TCCW conditions. Incubation was provided for 7 days under light conditions at 30 °C, 150 rpm rotation speed with an initial pH level of 8. Error bars indicate standard deviations over triplicate experiments with separately grown fungal cells.

**Figure 6** Pigment production by *F. graminearum* after incubation in A) 10^2 diluted TFCW, B) 10^2 diluted TCCW, C) 10^2 diluted deproteinized TFCW, D) 10^2 diluted deproteinized TCCW E) TFCW, F) TCCW, G) Deproteinized TFCW, H) Deproteinized TCCW. Incubation was performed for 7 days under light conditions at 30 °C and 150 rpm rotation speed with an initial pH level of 8. The reddish color indicates pigment production. TFCW: Turkish Feta Cheese Whey, TCCW: Turkish Cheddar Cheese Whey, DTFCW: Deproteinized Turkish Feta Cheese Whey, DTCCW: Deproteinized Turkish Cheddar Cheese Whey

Molasses is obtained from sugar cane as a byproduct of the white sugar production process and has been reported to contain polysaccharides, sucrose, glucose, fructose, amino acids, and some inorganic salts (Akar and Canbaz, 2016; Jiang et al., 2019). By comprising complex lignocellulosic carbon-containing compounds, molasses are also reported as a low-cost energy source for microbial growth and the production of microbial metabolites under different fermentation conditions (Hamano and Killikian, 2006; Mustafa et al., 2020). In line with this information, using molasses instead of glucose as a supplementary material has been reported as more advantageous for the red pigment production by *Monascus purpureus* AKH12 via solid state fermentation by offering a lower cost than using other sugars (Nimno et al., 2015). In our study, 10^3 times diluted molasses is also determined as a suitable growth media for reddish pigment production by *F. graminearum* (Fig. 4).

Whey is one of the major wastes generated by dairy industries and has been considered as an important environmental pollutant due to its high organic content for years (Güven et al., 2012; Hausjell et al., 2019; Mehri et al., 2021). Since whey is rich in organic ingredients such as carbon and nitrogen, it is regarded as a low-cost, robust, and sustainable microbial pigment production medium. Additionally, the utilization of whey with bioprocesses reduces environmental pollution. In this respect, similar to one of the recent studies that investigate the production of red color pigment from whey by using *Monascus purpureus* (Mehri et al., 2021), we aimed to test whether or not pigment production by *F. graminearum* is also possible when agro-industrial residues such as TFCW and TCCW are used as growth media in the last part of this study. According to our findings, although the growth of *F. graminearum* was significantly lower in 10^2 times diluted TFCW, TCCW, DTFCW, and DTCCW (p<0.001) (Fig. 5); more reddish pigment production by *F. graminearum* was visually observed in them, compared to their non-diluted versions (Fig. 6). Additionally, more pigment production by *F. graminearum* in deproteinized versions of non-diluted TFCW and TCCW were visually observed. Similar to our findings, the water-soluble yellow pigment has been reported to be produced from *Penicillium aculeatum* ATCC 10409 in submerged culture using a whey media (Afshari et al., 2015). Additionally, a recent study with pigment-producing fungal isolates from soil (including *Fusarium solani*) has reported pigment production by these isolates on green waste and whey cocktail media using submerged and solid-state fermentation methods (Molelekoa et al., 2021). To the best of our knowledge, this is the first report showing the reddish pigment production by *F. graminearum* using agro-industrial residues of molasses and whey. Fungal species, such as those belonging to the *Fusarium* genus, have been found to have the capability to include their use in food products, beverages, cosmetics, pharmaceuticals, and textile dying (Lopes et al., 2013). However, it is essential to consider the possible risk of mycotoxins associated with bio-colorants derived from fungal sources as well (Lopes et al., 2013; Pessôa et al., 2017; Cambaza et al., 2018).
produce mycocyanins, which are toxic secondary metabolites (Pessina et al., 2017). Therefore, the detection of mycocyanins in bio-colorants is crucial to ensure consumer safety and compliance with regulatory standards.

CONCLUSION

In conclusion, this study demonstrates that the reddish F. graminearum pigment obtained in this study is antimicrobial against two important food pathogens of B. cereus and S. aureus. Additionally, an initial pH level of 8.1, 150 rpm rotation speed, 30°C temperature, and 9 days of incubation under the light condition in Malt Extract Broth (MEB) are the optimum growth conditions for this reddish pigment production at maximum levels. Moreover, the utilization of agro-industrial residues such as molasses and whey as cost-effective growth media has been successfully determined. These findings highlight the potential of this reddish pigment as an antimicrobial bio-colorant for the food industry and the conversion of industrial residues into valuable bioproducts. However, it is important to conduct further research, including safety assessment, to explore the applicability of this pigment in diverse food applications.

Acknowledgment: We would like to thank Dr. Hanife Guler Donmez from Hacettepe University for the statistical analysis and Dr. Sezen Bilen Ozyurek from Hacettepe University for providing us the Bacillus cereus isolate.

Funding: This research belongs to the master thesis of the first author and the authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Conflicts of Interest: The authors declare that there are no conflicts of interest.

Authors’ Contribution

Conceptualization: TD and ISB and GS; Supervision: ISB; Investigation: TD; Data analysis: TD, GS, and ISB; Writing – first draft: TD and GS; Review and editing: GS and ISB. All authors have read and agreed to the published version of the manuscript.

REFERENCES


