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RESEARCH PAPER

Water-Soluble Red Pigment Production by *Paecilomyces sinclairii* and Biological Characterization

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Abstract Natural pigments have several advantages over synthetic colorants. In this study, the production of red pigment produced by *Paecilomyces sinclairii* in microbial fermentation was demonstrated and the pigment was purified and characterized. The red pigment was produced from submerged fungal fermentation and fractionated by medium pressure flash chromatography. After fractionation, the spectrophotometric characterization of the red pigment revealed an λ_{max} at 520 nm. Antimicrobial activity of the red pigment fraction was also studied against *Escherichia coli* O157 and *Pseudomonas aeruginosa* PAO1. The fraction (F2-F6) of the red pigment exhibited broad-spectrum antimicrobial activity in both bacteria. These results demonstrate the potential of this pigment in inhibiting bacterial growth and in food processing and other food-related applications.

Keywords: natural pigment, *Paecilomyces sinclairii*, antimicrobial activity, fractionation, chemical structure

1. Introduction

Historically, the use of natural pigments has attracted great interest as a food additive, medicinal compound, and dye in the textile and cosmetic industries, and in the preparation of alcoholic beverages. These uses reflect beneficial effects of natural pigments, such as heat stable behavior and antimicrobial activity, compared to synthetic or artificial colorants [1]. Natural pigments can be produced from two major processes: extraction from plants and by microbial fermentation from various bacteria [2]. Microbial fermentation from various microorganisms to produce pigments has many advantages over extraction from plants. One of the key advantages is the light stability and antimicrobial activity of different pigments, and their use in fermentation strategies.

A number of microorganisms produce several pigments with high production yield. These include *Hahella chejuensis* [3], *Monascus* [4,5], *Cordyceps unilateralis* [2], *Paecilomyces sinclairii* [1,6], *Serratia macescens* [7], *Spirillospora* [8], *Pantoea agglomerans* [9], and many others [10-14]. Among them, the production of red pigments from *Monascus*-mediated fermentation has been widely studied and well-characterized by spectroscopy and mass spectrometry [15-18]. Many studies have demonstrated for the optimal culture conditions that allow the high production of red pigments during submerged or solid fermentation [1,6,16, 17]. Most microbial pigments have pharmacological activities, such as antimicrobial, anti-cancer, and immuno-suppressive activities [14,19].

We previously found that the basidiomycete fungus, *P. sinclairii*, produces red pigment with broad spectrum activity and high production yield under optimal submerged culture conditions [6]. We were interested in identifying the antimicrobial activity and structural property such as molecular mass. In this study, we aimed to elucidate the antimicrobial activity of the red pigment produced by submerged cultures of *P. sinclairii* and to identify their molecular functions by mass spectroscopy.

2. Materials and Methods

2.1. Microorganisms, media, and chemicals

The fungal strain *P. sinclairii* was used for the production

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of the red pigment. The stock culture was maintained on potato dextrose agar (PDA) slants. The slants were inoculated, incubated at 25°C for 7 days, and then stored at 4°C. The seed culture medium comprised yeast extract (3 g/L), malt extract (3 g/L), peptone (5 g/L), and glucose (10 g/L) in distilled water. Ethyl acetate, water, hexane, and acetonitrile were obtained from Sigma-Aldrich (St. Louis, MO, USA). PDA, malt extract and peptone were purchased from Difco Laboratories (Detroit, MI, USA). Monascus pigment as standard was purchased from Wako Chemical Ltd. (Tokyo, Japan). All other reagents and solvents used were analytical grade unless stated otherwise.

2.2. Culture conditions and microbial production of red pigment

P. sinclairii was initially grown on PDA medium in a Petri dish and transferred to the seed culture medium by punching out 5-mm discs of the agar plate culture with a sterilized cutter. The seed culture was grown in 250-mL flasks containing 50 mL seed culture medium at 25°C on a rotary shaker at 150 rpm for 2 days. Fermentations for the production of the red pigment were carried out in 250-mL flasks containing 100 mL basal medium at 25°C on a rotary shaker at 150 rpm for 5 days.

2.3. Purification of red pigment

Final culture broth containing red pigment was extracted successively as described previously [1]. The water-soluble pigment was dried in a freeze dryer for 3 days and then used for fractionation of the red pigment. The crude red pigment was separated using medium pressure flash chromatography (Reveleris X2; Grace, Buchi) over silica gel using an ethyl acetate/hexane/water/acetonitrile step gradient. Fractions of 25 mL were collected. Compounds were detected using an evaporative light scattering detector at 201, 254, and 573 nm.

2.4. Antimicrobial activity test

Escherichia coli O157 and *Pseudomonas aeruginosa* PAO1 were used to test antimicrobial activity of the purified red pigment. The bacteria were each grown in LB medium for 5 h to reach at exponential period of growth. Bacteria were transferred into wells of a 96-well microtiter plate. Ten-microliter aliquot of purified red pigment was added to each well. The microtiter plates were incubated at 37°C for 12 h, and the absorbance was read at 595 nm every 60 min using an Infinite® F50 microplate reader (Tecan, Männedorf, Switzerland). Control wells were incubated without addition of red pigment.

2.5. Analytical methods

The maximum absorbance of red pigment was determined by ultraviolet-visible (UV-Vis) spectroscopy (Shimadzu, Kyoto, Japan). The samples were analyzed by electrospray ionization mass spectrometry (ESI-MS) using a SYNAPT G2 apparatus (Waters, Elstree, UK) to determine the molecular weight of the red pigment. In detail, ESI-MS analysis was performed using a time-of-flight (TOF) mass spectrometer equipped with an ESI source. The mass spectrometer was operated in full ion scanning, which was chosen to detect positive ions at an m/z of 305.1. For full scanning, the mass range was set at an m/z 50 ~ 2,000. The elemental compositions from the accurate mass measurements of m/z values.

3. Results and Discussion

3.1. Fractionation of red pigment produced by submerged fungal fermentation

We reported the first example of the production of a red pigment from submerged fermentation by *P. sinclairii* [1]. We suspected that the red pigment might have antibacterial activity since a number of other natural pigments had been demonstrated to possess attractive pharmacological capabilities including antibacterial activity. To explore this possibility, red pigment from submerged fermentation were produced as described previously [1,6]. The absorption spectra were measured by UV-Vis spectroscopy. Fig. 1 shows the absorption spectra of the red pigment in water. Their maximum absorption wave was observed at 520 nm, similar to that reported in the literature [20,21]. Thus, the major spectra of the red pigment were classified as water-soluble red pigment. In addition, the color of the red pigment

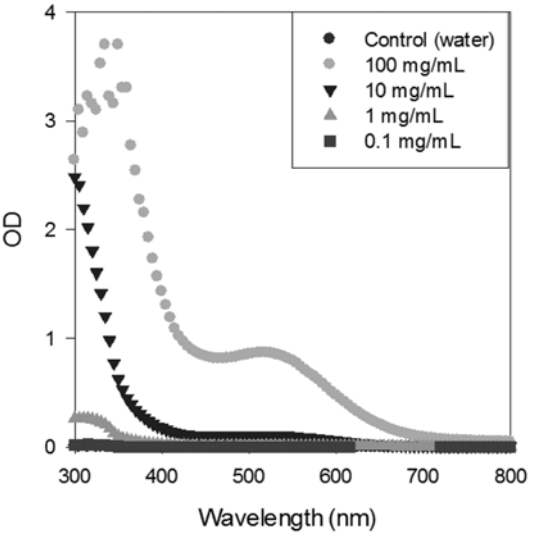

Fig. 1. Visible absorption spectra of the red pigment in submerged fungal fermentation. The maximum absorbance of the pigment was determined by UV-Vis spectroscopy.

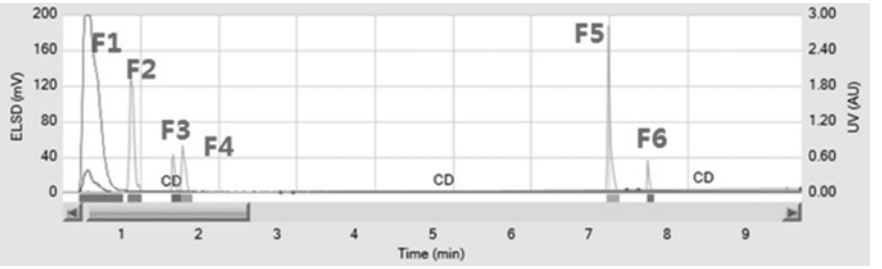

Fig. 2. Medium pressure flash chromatography chromatogram of the red pigment.

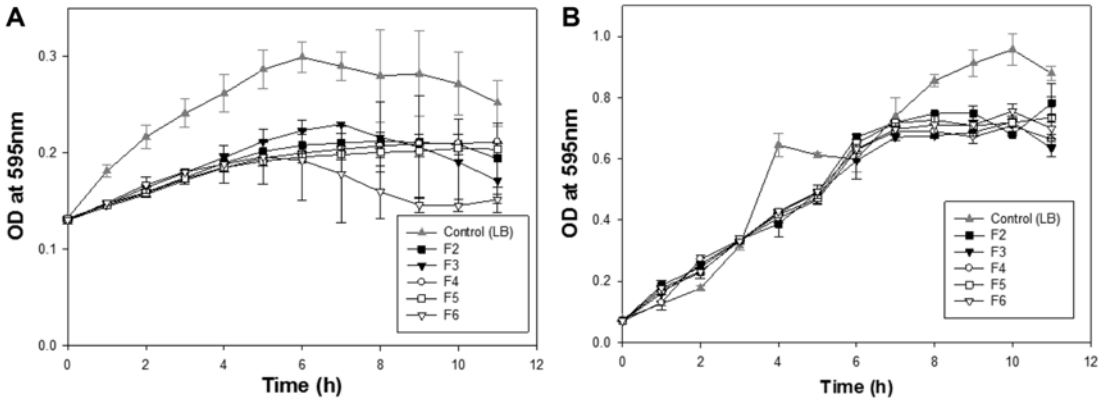

Fig. 3. Antimicrobial activity of red pigment produced by *P. sinclairii* against *E. coli* O157 (A) and *P. aeruginosa* PAO1 (B). ▲; control, ■; F2 fraction, ▼; F3 fraction, ○; F4 fraction, □; F5 fraction, ▽; F6 fraction.

changed depending on pH. This was consistent with our previous report [1] and other results [21], indicating that both red pigments produced by *P. sinclairii* and *Isaria farinosa* are similar in chemical structure. We fractionated the red pigment using medium pressure flash chromatography to study the biological activity, as shown in Fig. 2. A total of five fractions (F2-F6, excluding F1) were obtained and their antimicrobial activity was examined.

3.2. Antimicrobial activity

As an initial measurement of antimicrobial activity, the aforementioned five fractions obtained by medium pressure flash chromatography (MPLC) purification were tested against *E. coli* O157 and *P. aeruginosa* PAO1. Fig. 3 depicts the antimicrobial activity of red pigment produced by *P. sinclairii* in the presence of each fraction (F2-F6) in the culture medium. The fractions displayed antimicrobial activity against *E. coli* O157 compared to the control (Fig. 3A). Fractions F2, F4, and F5 displayed similar and slightly less antimicrobial activities than F6. The activity of F6 was most pronounced as a function of culture time. In addition, most of the fractions (F2-F6) also showed antimicrobial activity against *P. aeruginosa* PAO1. As shown in Fig. 3B, the red pigment displayed an antimicrobial effect on the growth of bacteria, with the inhibitory effect increasing with the increased concentration of the red pigment (data not shown). The results showed that the red pigment has broad-spectrum activity. More interestingly, it was also concluded that *P. aeruginosa* PAO1 was more sensitive to the red pigment compared to *E. coli* O157. *P. aeruginosa* is an opportunistic pathogen that can display resistance to antibiotics. It may deleteriously affect patients because of the production of virulence factors. The red pigment produced by *P. sinclairii* may serve as an alternative for new antibiotic development in clinically relevant bacterial pathogens. Therefore, we tried to further characterize the chemical structure of the F3 fraction, which had higher antimicrobial activity against *P. aeruginosa* PAO1.

3.3. Chemical structure characterization of red pigment

In our initial experiments described above, the F3 fraction showed the best antimicrobial activity for *P. aeruginosa*. Thus, we wanted to determine their chemical structure by electrospray ionization mass spectrometry mass spectroscopy (ESI-MS). ESI-MS of the red pigment demonstrated a large peak at *m/z* 305.13 (Fig. 4), consistent with the $(M+H)^+$ ion of the red pigment. Velmurugan *et al.* [21] reported that the large peak of red pigment produced by *I. farinosa* was positioned at *m/z* 256. They suggested that both red pigments from different microorganism have a similar color variation, depending on the pH. Assuming that the red pigment from *P. sinclairii* used in this study

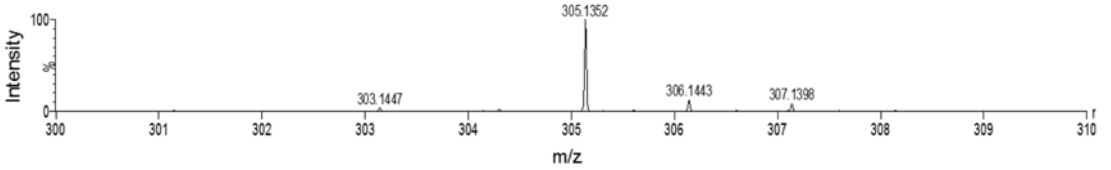

Fig. 4. Mass spectrometry spectrum profile of the fractionation (F3 fraction) of the red pigment produced by *P. sinclairii.*

retained a different chemical structure, the data indicate that this is the first example of red pigment having antimicrobial activity. Unfortunately, further experiments using nuclear magnetic resonance and comparison with a chemical library were not enough to fully conclude the nature of the red pigment. Further investigations are in progress to fully understand the red pigment by nuclear magnetic resonance analysis and infrared spectrometry.

4. Conclusion

In conclusion, we demonstrated the first example of production and biological characterization of red pigment produced from *P. sinclairii.* This water-soluble red pigment showed broad spectrum antimicrobial activity and structural different property over conventional synthetic colorant or natural pigment from different bacteria. The results of this study suggest the potential of this pigment as alternative colorant and food preservative's in food processing or other food-related industry.

Acknowledgement

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References

1. Cho, Y. J., J. P. Park, H. J. Hwang, S. W. Kim, J. W. Choi, and J. W. Yun (2002) Production of red pigment by submerged culture of *Paecilomyces sinclairii*. *Lett. Appl. Microbiol*. 35: 195-202.
2. Unagul, P., P. Wongsa, P. Kittakoop, S. Intamas, P. Srikitikulchai, and M. Tanticharoen (2005) Production of red pigments by the insect pathogenic fungus *Cordyceps unilateralis* BCC 1869. *J. Ind. Microbiol. Biotechnol*. 32: 135-140.
3. Kim, D., J. S. Lee, Y. K. Park, J. F. Kim, H. Jeong, T. K. Oh, B. S. Kim, and C. H. Lee (2007) Biosynthesis of antibiotic prodiginines in the marine bacterium Hahella chejuensis KCTC 2396. *J. Appl. Microbiol.* 102: 937-944.
4. Jung, H., C. Kim, K. Kim, and C. S. Shin (2003) Color characteristics of monascus pigments derived by fermentation with various amino acids. *J. Agric. Food. Chem*. 51: 1302-1306.
5. Kim, C., H. Jung, J. H. Kim, and C. S. Shin (2006) Effect of monascus pigment derivatives on the electrophoretic mobility of bacteria, and the cell adsorption and antibacterial activities of pigments. *Colloids Surf. B: Biointerf.* 47: 153-159.
6. Cho, Y. J., H. J. Hwang, S. W. Kim, C. H. Song, and J. W. Yun (2002) Effect of carbon source and aeration rate on broth rheology and fungal morphology during red pigment production by *Paecilomyces sinclairii* in a batch bioreactor. *J. Biotechnol*. 95: 13-23.
7. de Araujo, H. W., K. Fukushima, and G. M. Takaki (2010) Prodigiosin production by *Serratia marcescens* UCP 1549 using renewable-resources as a low cost substrate. *Molecules* 15: 6931-6940.
8. Hacene, H. and G. Lefebvre (1996) HP17, a new pigment-like antibiotic produced by a new strain of Spirillospora. *J. Appl. Bacteriol*. 80: 565-569.
9. Fujikawa, H. and R. Akimoto (2011) A new blue pigment produced by *Pantoea agglomerans* and its production characteristics at various temperatures. *Appl. Environ. Microbiol*. 77: 172-178.
10. Schewe, H., A. Kreutzer, I. Schmidt, C. Schubert, and J. Schrader (2017) High concentrations of biotechnologically produced astaxanthin by lowering pH in a *Phaffia rhodozyma* bioprocess. *Biotechnol. Bioproc. Eng*. 22: 319-326.
11. Liu, G. Y. and V. Nizet (2009) Color me bad: microbial pigments as virulence factors. *Trends Microbiol*. 17: 406-413.
12. Jeon, J. -Y., S. -Y. Kim, H. -Y. Kim, S. -H. Kim, B. -J. Lee, S. R. Lim, and H. -K. Choi (2018) Effects of agitating culture condition on the growth, metabolic and carotenoid profiles of *Lemna paucicostata*. *Biotechnol. Bioproc. Eng*. 23: 23-30.
13. Yun, M., Y. -K. Oh, R. Praveenkumar, Y. -S. Seo, and S. Cho (2017) Contaminated bacterial effects and qPCR application to monitor a specific bacterium in *Chlorella* sp. KR-1 culture. *Biotechnol. Bioproc. Eng*. 22: 150-160.
14. Zhou, Z. Y. and J. K. Liu (2010) Pigments of fungi (macromycetes). *Nat. Prod. Rep*. 27: 1531-1570.
15. Kim, C., H. Jung, Y. O. Kim, and C. S. Shin (2006) Antimicrobial activities of amino acid derivatives of monascus pigments. *FEMS Microbiol. Lett*. 264: 117-124.
16. Kongruang, S. (2011) Growth kinetics of biopigment production by Thai isolated Monascus purpureus in a stirred tank bioreactor. *J. Ind. Microbiol. Biotechnol*. 38: 93-99.
17. Loret, M. O. and S. Morel (2010) Isolation and structural characterization of two new metabolites from monascus. *J. Agric. Food. Chem*. 58: 1800-1803.
18. Babitha, S., C. R. Soccol, and A. Pandey (2007) Effect of stress on growth, pigment production and morphology of *Monascus* sp. in solid cultures. *J. Basic Microbiol*. 47: 118-126.
19. Perez-Tomas, R., B. Montaner, E. Llagostera, and V. Soto-Cerrato (2003) The prodigiosins, proapoptotic drugs with anticancer properties. *Biochem. Pharmacol*. 66: 1447-1452.
20. Lebeau, J., M. Venkatachalam, M. Fouillaud, T. Petit, F. Vinale, L. Dufosse, and Y. Caro (2017) Production and new extraction method of polyketide red pigments produced by Ascomycetous fungi from terrestrial and marine habitats. *J. Fungi* 3: 34-55.
21. Velmurugan, P., Y. H. Lee, K. Nanthakumar, S. Kamala-Kannan, L. Dufossé, S. A. S. Mapari, and B. -T. Oh (2010) Water-soluble red pigments from *Isaria farinosa* and structural characterization of the main colored component. *J. Basic Microbiol*. 50: 581-590.