J. Microbiol. Biotechnol. (2005), **15**(4), 891–894

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

© The Korean Society for Microbiology and Biotechnology

Terrein, a Melanin Biosynthesis Inhibitor, from *Penicillium* sp. 20135

KIM, WON-GON, IN-JA RYOO, SEO-HYOUNG PARK[1], DONG-SEOK KIM[1], SANGKU LEE, KYOUNG-CHAN PARK[1], AND ICK-DONG YOO*

Korea Research Institute of Bioscience and Biotechnology, P.O. Box 115, Yusong, Daejeon 305-600, Korea
[1]Department of Dermatology, Seoul National University College of Medicine, 28 Yongon-Dong, Chongno-Gu, Seoul 110-744, Korea

Received: October 26, 2004
Accepted: November 24, 2004

Abstract In the course of screening a melanin biosynthesis inhibitor, terrein, 4,5-dihydroxy-3-propenyl-2-cyclopenten-1-one, was isolated from *Penicillium* sp. Terrein was found to have a strong inhibitory activity on melanin formation in B16 melanoma and melanocyte Mel-Ab cells.

Key words: Terrein, melanin, *Penicillium*

Melanin is biosynthesized in the melanosomes of melanocytes, moved to keratinocytes, and stored in the epidermis [18, 19]. Hyperpigmentation, such as chloasma, coloration, or freckles, implies abnormally increased amount of melanin in the epidermis. Melanogenesis consists of two rate-limiting reactions catalyzed by tyrosinase; i.e., the hydroxylation of tyrosine to yield 3, 4-dihydroxyphenylalanine (DOPA) and the subsequent oxidation of DOPA to dopaquinone [5]. Thus, melanin biosynthesis inhibitors are useful as skin-whitening agents in cosmetics.

Fungi and actinomycetes provide many important biomedical substances [6, 7, 12, 17, 21]. Kojic acid, currently used as a cosmetic agent for the purpose of skin whitening, was isolated from *Aspergillus oryzae* [1]. Stronger and safer skin-whitening agents, however, are needed, because of a carcinogenic potential as well as a weak whitening effect of kojic acid [3, 20]. In order to discover new classes of cosmetic agents, we have screened melanin biosynthesis inhibitors from microbial metabolites and found strong tyrosinase inhibitors, melanocins A-C, produced by *Eupenicillium shearii* [9, 10]. In the course of our continuous search for melanin biosynthesis inhibitors using melanocyte Mel-Ab cells, terrein was newly isolated from *Penicillium* sp. 20135. It has a relatively simple structure [4]. However, there are only a few reports about terrein, and its effects are hardly recognized. In the present study, we describe the purification and identification of a melanin biosynthesis inhibitor produced by *Penicillium* sp. 20135 and its melanin biosynthesis inhibitory activity.

The fungal strain 20135 was isolated from a soil sample that was collected in a corn field around Sokcho-city, Kangwon-do, Korea, and assigned to the *Penicillium* sp. 20135. Fermentation was carried out in a liquid culture medium containing 2% glucose, 0.2% yeast extract, 0.5% polypeptone, 0.05% $MgSO_4$, and 0.1% KH_2PO_4 (pH 5.7 before sterilization). A piece of the strain 20135 from a mature plate culture was inoculated into a 500-ml Erlenmeyer flask containing 80 ml of the above steril seed liquid medium and cultured on a rotary shaker (150 rpm) at 28°C for 3 days. For the production of terrein, 5 ml of the seed culture were transferred into one-liter Erlenmeyer flasks (15 flasks) containing 100 ml of the above medium, and cultivated for 6 days under the same conditions. The culture broth (1 l) was extracted with 80% acetone, and the extract was concentrated *in vacuo* to an aqueous solution, which was then extracted three times with an equal volume of ethyl acetate (EtOAc). EtOAc extract was concentrated *in vacuo* to dryness. The crude extract was subjected to SiO_2 (Merck Art No. 7734.9025) column chromatography, followed by stepwise elution with $CHCl_3$-methanol (MeOH) (50:1, 20:1, 10:1). The active fractions eluted with $CHCl_3$-MeOH (20:1) were pooled and concentrated *in vacuo*. The residue was finally applied to a Sephadex LH-20 column and the column was then eluted with MeOH to afford the purified compound (15 mg). The purity of the compound was found to be more than 99% by high-performance liquid chromatography with an ODS column (YMC C_{18}) eluted with MeOH:H_2O (30:70). The molecular weight of the active compound was determined to be 154.0 by its ESI mass spectrum having m/z 155.03 $(M+H)^+$ and 177.00 $(M+Na)^+$. Its ^1H and ^{13}C NMR spectral data were as follows; δ_H [CD_3OD] 6.81 (1H, dq, J=6.8, 15.8 Hz, H-2'), 6.44 (1H,

*Corresponding author
Phone: 82-42-860-4330; Fax: 82-42-860-4595;
E-mail: idyoo@kribb.re.kr

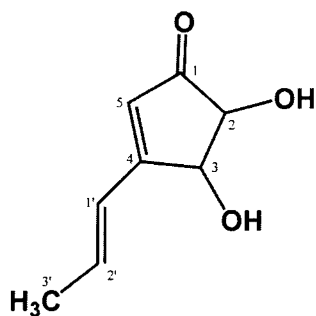

Fig. 1. The structure of terrein.

dd, J=1.5, 15.8 Hz , H-1'), 6.0 (1H, s, H-5), 4.67 (1H, d, J=2.8 Hz, H-3), 4.07 (1H, d, J=2.8 Hz, H-2), and 1.94 (3H, d, J=1.5, 6.8 Hz, H-3'); δ_C [CD_3OD] 205.5 (C-1), 170.0 (C-4), 145.1 (C-2'), 126.0 (C-1'), 125.1 (C-5), 82.5 (C-2), 78.0 (C-3), and 19.2 (C-3'). Based on electron spray ionization-mass, ^{1}H-NMR, and ^{13}C-NMR, heteronuclear multiple quantum coherence, and heteronuclear multiple-bond correlation spectral data, the purified compound was verified as terrein, 4,5-dihydroxy-3-propenyl-2-cyclopenten-1-one ($C_8H_{10}O_3$, mol, wt 154 kDa) (Fig. 1) [11]. Since the optical rotation value of the purified terrein was zero in MeOH, the compound was found to be a racemic form.

Mel-Ab is a mouse-derived spontaneously immortalized melanocyte cell line that produces a large amount of melanin [2]. In the present study, Mel-Ab cells were cultured in Dulbecco's Minimal Essential Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 nM 12-O-tetradecanoylphorbol-13-acetate (TPA), 1 nM cholera toxin (CT), 50 µg/ml streptomycin, and 50 U/ml penicillin at 37°C in 5% CO_2. On the other hand, B16/F10 murine melanoma cells were incubated in DMEM supplemented with 10% FBS, 50 µg/ml streptomycin, and 50 U/ml penicillin at 37°C in a 5% CO_2 atmosphere.

α-Melanocyte stimulating hormone (α-MSH) has been reported to increase melanin production [15]. Thus, we determined the effects of terrein on the α-MSH-mediated stimulation of melanogenesis in B16 melanoma cells, and found that 1 µM α-MSH increased the amount of melanin detected in the extracellular media, as measured by spectrophotometry, after 4-days α-MSH of treatment. Addition of increasing concentration of terrein, however, decreased extracellular melanin release induced by α-MSH treatment (Fig. 2): Terrein at 10 µM inhibited α-MSH-induced melanogenesis by about 50%.

Effects of terrein on melanin formation in melanocyte Mel-Ab cell line that produces a large amount of melanin were evaluated. When treated at the concentrations of 0.25 and 50 µM for 4 days, terrein-treated cells were much less pigmented than the untreated cells. The melanin contents of Mel-Ab cells were assayed spectrophotometrically after the treatment with terrein for 4 days. In agreement with the microscopic observations, melanin levels were strongly reduced in a dose-dependent manner (Fig. 3). Terrein showed a potency similar to that of phenyl thiocarbamide (PTU), as a control, which inhibited melanin synthesis by 60% at 50 µM. Terrein showed stronger activity than kojic acid, which inhibited melanin synthesis by 20% at 100 µM. To determine whether terrein had a cytotoxic effect on Mel-Ab cells, the cells were treated with up to 100 µM terrein for 24 h and their viability was measured using crystal violet assay. However, terrein had no cytotoxic effect on Mel-Ab cells at the concentrations used.

Many skin-whitening agents inhibit tyrosinase directly. Thus, to investigate any possible direct effect of terrein on tyrosinase, we measured the activity of mushroom tyrosinase by the method previously reported [9]. Interestingly, terrein

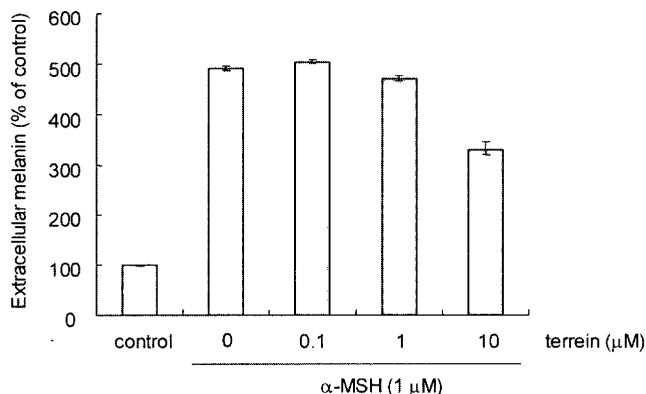

Fig. 2. Effects of terrein on extracellular melanin production in B16 melanoma cells.

B16 cells were treated with 0.1–10 µM terrein in the presence of 1 µM α-MSH for 4 days. Absorbance at 400 nm was measured and expressd as a percentage of basal melanin released per cell. Each determination was made in triplicate and data shown are mean±SD.

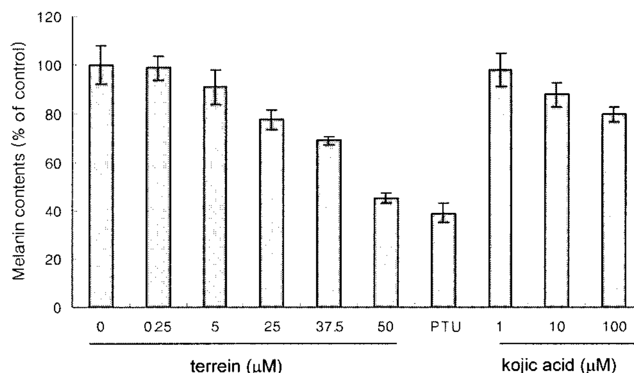

Fig. 3. Effects of terrein on melanogenesis in Mel-Ab cells.

Mel-Ab cells were cultured for 4 days in the medium containing 0.25–50 µM terrein. The melanin contents in the crude cell extracts were spectrophotometrically assayed at 400 nm. Phenyl thiocarbamide (50 µM) was used as a control. Each determination was made in triplicate and data shown are mean±SD.

showed no inhibitory activity against mushroom tyrosinase, even at 200 μM concentration. To find out whether terrein affected tyrosinase expression, the protein level of tyrosinase was examined by Western blot analysis. The reduction of the amount of tyrosinase was detected in Mel-Ab cells after terrein treatment (data not shown). More detailed mechanism on the hypopigmenting effect of terrein will be reported elsewhere.

Terrein was first isolated as a metabolite of *Aspergillus terreus* Thom in 1935 by Raistrick and has also been reported to be produced by other species of *Aspergillus* and *Penicillium raistrickii* [8]. It is known that terrein has weak antibacterial activity against *Escherichia coli*, *Entercoccus faecalis*, *Bacillus subtilis*, and *Staphylococcus aureus* [13]. Terrein is an intermediate during the preparation of prostaglandins of the C series and analogs of other prostaglandins [14]. Even though terrein was known long ago, its biological activitiy has hardly been studied, except for its antimicrobial effects [13, 16]. In the present study, we found that terrein had an inhibitory effect on melanogenesis without a cytotoxic activity in cell-based assay. Importantly, the skin-whitening effect of terrein was also demonstrated in our preliminary clinical test (data not shown). Thus, terrein, a relatively small metabolite isolated from *Penicillium* sp., has great potential as an alternative for the skin-whitening agents currently used.

Acknowledgment

This work was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A-050432).

REFERENCES

1. Cabanes, J., S. Chazarra, and F. Garcia-Carmona. 1994. Kojic acid, a cosmetic skin whitening agent, is a slow binding inhibitor of catecholase activity of tyrosinase. *J. Pharm. Pharmacol.* **46:** 982–985.
2. Dooley, T. P., R. C. Gadwood, K. Kilgore, and L. M. Thomasco. 1994. Development of an *in vitro* primary screen for skin depigmentation and antimelanoma agents. *Skin Pharmacol.* **7:** 188–200.
3. Fujimoto, N., H. Watanabe, T. Nakatani, G. Roy, and A. Ito. 1998. Induction of thyroid tumors in (C57BL/6N x C3H/N)F1 mice by oral administration of kojic acid. *Food Chem. Toxicol.* **36:** 697–703.
4. Harper, J. K., A. E. Mulgrew, J. Y. Li, D. H. Barich, G. A. Strobel, and D. M. Grant. 2001. Characterization of stereochemistry and molecular conformation using solid-state NMR tensors. *J. Am. Chem. Soc.* **123:** 9837–9842.
5. Hearing, V. J. and M. Jimenez. 1989. Analysis of mammalian pigmentation at the molecular level. *Pigment Cell Res.* **2:** 75–85.
6. Hyeon, J. D., K. D. Park, S. H. Kim, K. R. Kim, S. W. Choi, J. T. Kim, K. H. Choi, and J. H. Kim. 2004. Identification of *Streptomyces* sp. producing antibiotics against phytopathogenic fungi, and its structure. *J. Microbiol. Biotechnol.* **14:** 212–215.
7. Hwang, S. E., H. M. Im, D. H. Kim, H. J. Shin, D. H. Shin, J. E. Park, I. H. Jo, C. J. Kim, J. S. Yoo, J. M. Kang, D. Y. Lim, S. M. Ahn Jo, and H. J. Kwon. 2003. Isolation of *Streptomyces* sp. KK565 as a producer of β-amyloid aggregation inhibitor. *J. Microbiol. Biotechnol.* **13:** 809–814.
8. Kawakubo, J., H. Nishira, K. Aoki, and R. Shinke. 1994. Production of phenolic compounds by *Aspergillus* S-4 in sake cake medium and identification of terrein. *Biosci. Biotech. Biochem.* **57:** 1208–1209.
9. Kim, J.-P., B.-K. Kim, B.-S. Yun, I.-J. Ryoo, C.-H. Lee, I.-K. Lee, W.-G. Kim, S. Lee, Y.-R. Pyun, and I.-D. Yoo. 2003. Melanocins A, B and C, new melanin synthesis inhibitors produced by *Eupenicillium shearii*. I. Taxonomy, fermentation, isolation and biological properties. *J. Antibiot.* **56:** 993–999.
10. Kim, J.-P., B.-K. Kim, B.-S. Yun, I.-J. Ryoo, I.-K. Lee, W.-G. Kim, S. Lee, Y.-R. Pyun, and I.-D. Yoo. 2003. Melanocins A, B and C, new melanin synthesis inhibitors produced by *Eupenicillium shearii*. I. Physico-chemical properties and structure elucidation. *J. Antibiot.* **56:** 1000–1003.
11. Kolb, H. C., H. Hoffmann, and R. Martin. 1990. A total synthesis of racemic and optically active terrein (trans-4,5-dihydroxy-3-[(E)-1-propenyl]-2-cyclopenten-1-one). *Tetrahedron: Asymmetry* **1:** 237–250.
12. Lim, H. Y., M. K. Kim, Y. H. Cho, J. M. Kim, Y. H. Lim, and C. H. Lee. 2004. Inhibition of cell cycle progression and induction of apoptosis in HeLa cells by HY558-1, a novel CDK inhibitor isolated from *Penicillium minioluteum* F558. *J. Microbiol. Biotechnol.* **14:** 978–984.
13. Malstrom, J., C. Christophersen, A. F. Barrero, J. E. Oltra, J. Justicia, and A. Rosales. 2002. Bioactive metabolites from a marine-derived strain of the fungus *Emericella variecolor. J. Nat. Prod.* **65:** 364–367.
14. Mitscher, L. A., G. W. Clark III, and G. H. Bokelman. 1978. Method for the preparation of prostaglandin intermediates from a mold metabolite. U. S. Patent 4103091 (Jul. 25, 1978).
15. Ohguchi, K., Y. Banno, Y. Akao, and Y. Nozawa. 2004. Involvement of phospholipase D1 in melanogenesis of mouse B16 melanoma cells. *J. Biol. Chem.* **279:** 3408–3412.
16. Raistrick, H. and G. Smith. 1935. Biochemistry of micro.ovrddot.okirganisms. XLII. The metabolic products of *Aspergillus terreus* Thom. A new mold metabolic product - terrein. *Biochem. J.* **29:** 606–611.
17. Rhee, K. H. 2003. Purification and identification of an antifungal agent from *Streptomyces* sp. KH-614 antagonistic to rice blast fungus, *Pyricularia oryzae. J. Microbiol. Biotechnol.* **13:** 984–988.

18. Schroder, J. M. 1995. Cytokine networks in the skin. *J. Invest. Dermatol.* **105:** 20S–24S.
19. Spritz, R. A. and V. J. Hearing. 1994. Genetic disorders of pigmentation. *Adv. Hum. Genet.* **22:** 1–45.
20. Takizawa, T., K. Mitsumori, T. Tamura, M. Nasu, M. Ueda, T. Imai, and M. Hirose. 2003. Hepatocellular tumor induction in heterozygous p53-deficient CBA mice by a 26-week dietary administration of kojic acid. *Toxicol. Sci.* **73:** 287–293.
21. Yi, Y. S., S. H. Kim, M. W. Kim, G. J. Choi, K. Y. Cho, J. K. Song, and Y. H. Lim. 2004. Antifungal activity of *Streptomyces* sp. against *Puccinia recondite* causing wheat leaf rust. *J. Microbiol. Biotechnol.* **14:** 422–425.