Kurasoins A and B, New Protein Farnesyltransferase Inhibitors Produced by *Paecilomyces* sp. FO-3684

I. Producing Strain, Fermentation, Isolation, and Biological Activities

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(Received for publication May 7, 1996)

Ras proteins have been shown to be post-translationally farnesylated on a specific carboxy-terminal cysteine by protein farnesyltransferase (PFTase). Inhibition of PFTase is expected to alter membrane localization and activation of Ras proteins[1]. In the course of screening for PFTase inhibitors of microbial origin, we have previously reported gliotoxins[2], pepticinnamins[3,4], and andrastins[5~7]. Additional new PFTase inhibitors, kurasoins A and B (**1** and **2**, Fig. 1), were found from the cultured broth of *Paecilomyces* sp. FO-3684.

In this paper, taxonomy of the producing strain and fermentation, isolation, physico-chemical properties, and biological activities of kurasoins are described.

Taxonomy of Producing Strain FO-3684

Strain FO-3684 was originally isolated from a soil sample collected at Kurashiki City, Okayama Prefecture, Japan. From the characteristics described below, the fungus was identified as a strain of *Paecilomyces* sp. For the identification of the fungus, potato-dextrose agar (PDA), yeast extract-soluble starch agar (YpSs), and corn meal agar (CMA) were used.

Colonies on CMA grew rather restrictedly, attaining a diameter of 20~25 mm and was colored in pearl after incubation for 14 days at 25°C. The reverse of colonies was pearl in color. No soluble pigment was produced.

Morphological observation was carried out under a microscope (Olympus Vanox-S AH-2). When the strain FO-3684 was grown on CMA for 14 days at 25°C, the phialide was born directly from substrate hyphae and was hyaline with no branching as shown in Fig. 2. The phialide was swollen and tapered gradually toward the apex, but the bottom was constricted. The conidia were ellipsoidal to oval and $3.5\sim4.5\times2.5\sim3.0\,\mu m$ in diameter and with smooth surface.

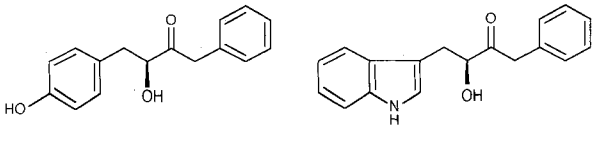
From the above characteristics, strain FO-3684 was identified as belonging to *Paecilomyces* sp.[8], and named *Paecilomyces* sp. FO-3684. This strain has been deposited at the National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology Japan, as FERM P-14761.

Production and Isolation

A stock culture of strain FO-3684 was inoculated into four 500-ml Erlenmeyer flasks containing 100 ml of a seed medium consisting of glucose 2.0%, yeast extract (Oriental Yeast Co.) 0.2%, $MgSO_4\cdot7H_2O$ 0.05%, Polypepton (Daigo Nutritive Chemicals) 0.5%, KH_2PO_4 0.1%, and agar 0.1%, at pH 6.0 before sterilization. Incubation was on a rotary shaker at 27°C for 3 days. Then 400 ml of the seed culture thus obtained was transferred into a 30-liter jar fermenter containing 20 liters of a production medium consisting of soluble starch (Wako Pure Chemical Ind.) 3.0%, glycerol 1.0%, soybean meals 2.0%, fermipan (Gist-brocades) 0.3%, KCl 0.3%, $CaCO_3$ 0.2%, $MgSO_4\cdot7H_2O$ 0.05%, and KH_2PO_4 0.05%, at pH 6.5 before sterilization. The fermentation was carried out at 27°C for 68 hours with agitation of 250 rpm and aeration of 10 liters/minute. A typical time course of the production of kurasoins is shown in Fig. 3.

The cultured broth (20 liters) was centrifuged and the mycelia were extracted with 18 liters of Me_2CO. The

Fig. 1. Structures of kurasoins A and B (**1** and **2**).



Kurasoin A (**1**) Kurasoin B (**2**)

Fig. 2. Photo micrograph of strain FO-3684 on corn meal agar (scale: 20 μm).

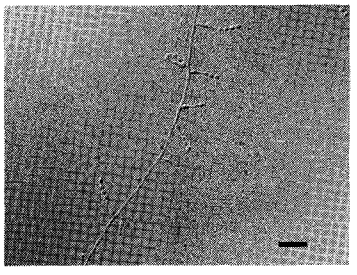


Fig. 3. A typical time course of the production of kurasoins.

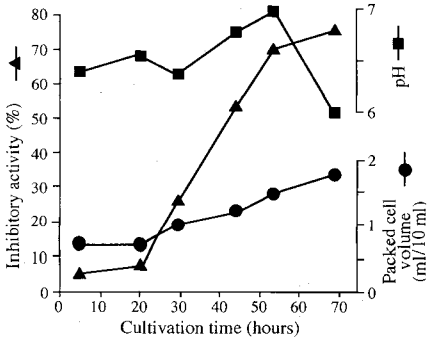


Table 1. Physico-chemical properties of **1** and **2**.

		1	2
Appearance		White powder	White powder
Molecular formula		$C_{16}H_{16}O_3$	$C_{18}H_{17}NO_2$
MW		256	279
HR-FAB-MS (*m/z*):	calcd	279.0997 $(M+Na)^+$	302.1157 $(M+Na)^+$
	found	279.1008 $(M+Na)^+$	302.1150 $(M+Na)^+$
UV λ_{max}^{MeOH} nm (ε)		206 (8350), 225 (sh, 5430), 278 (1230), 285 (sh, 870)	205 (sh, 14790), 218 (17020), 273 (3490), 283 (3630), 289 (3210)
IR ν_{max} (KBr) cm^{-1}		3380, 2920, 1710, 1600, 1520, 1500, 1450, 1340, 1050, 830, 820, 750	3360, 2920, 1710, 1500, 1460, 1340, 740, 700
Solubility:	soluble	MeOH, Me_2CO, EtOAc	MeOH, Me_2CO, EtOAc
	insoluble	H_2O, $CHCl_3$, Hexane	H_2O, $CHCl_3$, Hexane

extract was concd to 1 liter under reduced pressure. The residual soln was extracted twice with an equal vol of EtOAc. The extracts combined were concd under reduced pressure to give 18.8 g of brown oil. The oil was dissolved in 1 liter of MeOH. After washing twice with an equal vol of hexane, the MeOH soln was concd under reduced pressure to give 2.4 g of brown oil. The oil was dissolved in a small vol of MeOH, applied on a Sephadex LH-20 column (i.d. 2.8×97 cm, 600 ml, Pharmacia Biotech), and eluted with MeOH. The active fractions were concd under reduced pressure to give 56.1 mg of brown oil. The oil was further purified by HPLC under the following conditions: column, Senshu pak Pegasil ODS (i.d. 20×250 mm, Senshu Scientific Co.); mobile phase, 45% CH_3CN; flow rate, 8 ml/minute; detection, UV 205 nm. Compounds **1** and **2** were eluded at 19 and 41 minutes respectively under the above conditions. The eluates of HPLC were concd to remove CH_3CN, extracted with EtOAc at pH 3, and concd to dryness to give white powders of **1** (2.1 mg) and **2** (4.5 mg).

Physico-chemical Properties

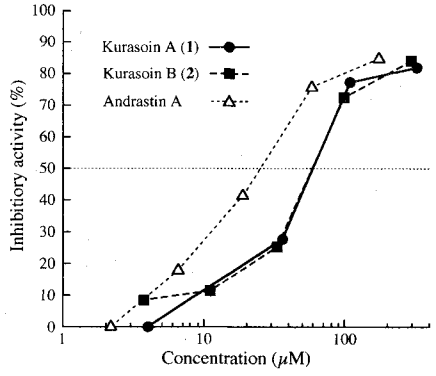
The physico-chemical properties of **1** and **2** are summarized in Table 1. The molecular formula of **1** and **2** were revealed by HR-FAB-MS as $C_{16}H_{16}O_3$ and $C_{18}H_{17}NO_2$, respectively. The IR spectra of both compounds exhibited characteristic absorptions at about 1710 cm^{-1}, suggesting the existence of a ketone group. The ^1H and ^{13}C NMR data as well as details of structure elucidations of **1** and **2** will be reported elsewhere[9].

Biological Properties

The PFTase inhibitory activity was measured as described previously[3]. As shown in Fig. 4, **1** and **2** inhibited PFTase in a dose-dependent manner. The IC_{50} values of **1** and **2** against PFTase were 59.0 and 58.7 μM, respectively (the IC_{50} value of andrastin A[6] was 25.7 μM at the same condition).

Compounds **1** and **2** showed no antimicrobial activity at 50 μg/disk (paper disk method) against *Bacillus subtilis* PCI 219, *Staphylococcus aureus* ATCC 6538p, *Micrococcus luteus* ATCC 9341, *Mycobacterium smegmatis* ATCC 607, *Escherichia coli* NIHJ, *Pseudomonas aeruginosa* PCI 602, *Xanthomonas campestris* pv. *oryzae* KB 88, *Bacteroides fragilis* ATCC 23745, *Acholeplasma laidlawii* PG 8, *Pyricularia oryzae* KF 180, *Aspergillus niger* ATCC 6275, *Mucor racemosus* IFO 4581, *Candida albicans* KF 1, and *Saccharomyces cerevisiae* KF 26.

Fig. 4. PFTase inhibitory activities of kurasoins.



Experimental

Mass spectrometry was conducted on a JEOL JMS-AX505 HA spectrometer. UV and IR spectra were measured with a Shimadzu UV-240 spectrophotometer and a Horiba FT-210 Fourier transform infrared spectrometer, respectively. Optical rotation was recorded on a JASCO model DIP-181 polarimeter. Melting point was measured with a Yanaco micro melting point apparatus MP-S3. NMR spectrum was obtained with JEOL JNM-EX270 spectrometer.

Acknowledgments

We wish to thank Ms. AKIKO NAKAGAWA and Ms. CHIKAKO SAKABE, School of Pharmaceutical Sciences, Kitasato University, for measurements of mass spectra. This work was supported in part by a grant from Ministry of Education, Science and Culture of Japan and Japan Keirin Association.

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