[Supplemental Information]

**Molecular cloning and characterization of type Ш polyketide synthase from *Plumbago zeylanica***

Seiichi Sakamotoa,†,\*, Yui Moritaa,†, Gorawit Yusakula,b, Waraporn Putalunc, Hiroyuki Tanakaa,d, Satoshi Morimotoa

a *Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*

b*School of Pharmacy, Walailak University, Thaiburi, Thasala, Nakhon Si Thammarat 80160, Thailand*

c *Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand*

d *Faculty of Pharmaceutical Sciences, Sanyo-Onoda City University, 1-1-1 Daigaku-dori, Sanyo-Onoda City 756-0884, Japan*

† These authors contributed equally to this work.

Running title: Functional analysis of type III PKSs from *P. zeylanica*

\*Corresponding author: Seiichi Sakamoto

Department of Pharmacognosy, Graduate School of Pharmaceutical Sciences,

Kyushu University; 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

E-mail: s.sakamoto@phar.kyushu-u.ac.jp Tel. & Fax: +81 92 642 6581

**Table S1**

Primers used for amplification of PKS1 and PKS2 cDNA fragments.

|  |  |
| --- | --- |
| 112K (Forward) | 5'- AAR GCI ITI AAR GAR TGG GGI CA -3' |
| 174A (Forward) | 5'- GCI AAR GAY ITI GCI GAR AAY AA -3' |
| 360T (Reverse) | 5'- CCC CAI TCI ARI CCI TCI CCI GTI GT -3' |
| 3'-GSP1 (Forward) | 5'-ACA AGC CTT ATT CGG AGA CG -3' |
| 3'-GSP2 (Forward) | 5'- CGA TCC TAG ACC AGG TTG AG -3' |
| Oligo dT-3sites Adaptor Primer  | 5'- CTG ATC TAG AGG TAC CGG ATC C -3' |
| 5'RT1 | 5'- GCA CAC AAG CGC TTG -3' |
| 5'RT2 | 5'- CGT GGA GAG GCC AAT -3' |
| 5'-GSP1 (Forward) | 5'- CAT CTC CTC AAG GAC GTT CC -3' |
| 5'-GSP2 (Forward) | 5'- CCA GGT TGA GGA GAA ACT CG -3' |
| 5'-GSP3 (Forward) | 5'- AGA ACA ACA AGG GAG CAC GAG -3' |
| 5'-GSP4 (Forward) | 5'- TTC AGG AGC CGT CAT CAT C -3' |
| 5'-GSP1 (Reverse) | 5'- CTC CTG AAC CGT CTC CGA AT -3' |
| 5'-GSP2 (Reverse) | 5'- ATC TCT GAG CAT ACG ACG AG -3' |
| 5'-GSP3 (Reverse) | 5'- GTC CTT CCG TCA AGC GAT TCA -3' |
| Full-length PKS2 (Forward) | 5'- CGC GAA TTC ATG GCC CCA TCT GTG GAA GAA-3' |
| Full-length PKS2 (Reverse) | 5'- TAT GCG GCC GCC TAG TTT ACA ACC GGG ACA CT-3' |



TAL

**(A)**

**(B)**





**(C)**



TAL

**(D)**

Figure S1 HPLC chromatograms of authentic TAL (A), purified PKS1 (B), substrate solution 1 (C), and reaction solution (D). Substrate solution 1 consists of acetyl-CoA and malonyl-CoA, and reaction solution consists of substrate solution 1 with purified PKS1. All solutions were prepared on the basis of 100 mM potassium phosphate buffer (pH 7.0). Solid arrow in (D) indicates the peak of TAL produced by PKS1.

**(A)**

Naringenin





**(B)**

**(C)**



Naringenin

**(D)**



Figure S2 HPLC chromatograms of authentic naringenin (A), purified PKS2 (B), substrate solution 2 (C), and reaction solution (D). Substrate solution 2 consists of consists of *p*-coumaroyl-CoA and malonyl-CoA, and reaction solution consists of substrate solution 2 with purified PKS2. All solutions were prepared on the basis of 100 mM potassium phosphate buffer (pH 7.0). Solid arrow in (D) indicates the peak of naringenin produced by PKS2.