Supplemental Materials

Identification of metabolites involved in the aerobic degradation of estrogen A/B-rings

Running title: Aerobic estrogen degradation pathway

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Table S1. UPLC-HRMS analysis of estrogen metabolites involved in aerobic estrone catabolism by strain SLCC.

Compound ID	UPLC behavior (RT[a], min)	Molecular formula/ (predicted molecular mass)[b]	Dominant ion peaks	Identification of product ions	Mode observed
estrone	8.11	$C_{18}H_{22}O_2$	253.16	$[M-H_2O+H]^+$	ESI and APCI
		270.16	271.17	$[M+H]^+$	ESI and APCI
pyridinestrone acid	3.99	$C_{18}H_{21}O_3N$	282.17	$[M-H_2O+H]^+$	ESI and APCI
		299.15	300.15	$[M+H]^+$	ESI and APCI
			322.14	$[M+Na]^+$	ESI
Compound Ie	5.58	$C_{16}H_{24}O_4$	245.16	$[M-2H_2O+H]^+$	ESI and APCI
		280.17	263.16	$[M-H_2O+H]^+$	ESI and APCI
			281.17	$[M+H]^+$	ESI and APCI
			303.15	$[M+Na]^+$	ESI
4-norestrogenic acid (Metabolite 5)*	5.90	$C_{17}H_{24}O_4$	257.15	$[M-2H_2O+H]^+$	APCI
		292.17	275.17	$[M-H_2O+H]^+$	ESI and APCI
			293.17	$[M+H]^+$	ESI and APCI
			315.16	$[M+Na]^+$	ESI
metabolite 7*	6.22	$C_{17}H_{24}O_5$	273.15	$[M-2H_2O+H]^+$	APCI
		308.16	291.16	$[M-H_2O+H]^+$	ESI and APCI
			309.17	$[M+H]^+$	ESI and APCI
			331.15	$[M+Na]^+$	ESI
metabolite 10*	5.03	$C_{15}H_{22}O_5$	247.14	$[M-2H_2O+H]^+$	ESI and APCI
		282.15	265.15	$[M-H_2O+H]^+$	ESI and APCI
			283.15	$[M+H]^+$	ESI and APCI
			305.14	$[M+Na]^+$	ESI
metabolite 11*	5.44	$C_{15}H_{20}O_5$	245.13	$[M-2H_2O+H]^+$	APCI
		280.13	263.14	$[M-H_2O+H]^+$	ESI and APCI
			281.14	$[M+H]^+$	ESI and APCI
			303.13	$[M+Na]^+$	ESI
metabolite 12*	5.15	$C_{15}H_{22}O_6$	263.15	$[M-2H_2O+H]^+$	ESI and APCI
		298.14	281.16	$[M-H_2O+H]^+$	ESI and APCI
			299.15	$[M+H]^+$	ESI and APCI
HIP	3.75	$C_{13}H_{18}O_4$	221.12	$[M-H_2O+H]^+$	ESI and APCI
		238.12	239.13	$[M+H]^+$	ESI and APCI
			261.11	$[M+Na]^+$	ESI

[a]RT, retention time. [b]The predicated molecular mass was calculated using the atom mass of ^{12}C (12.00), ^{16}O (15.99), and ^{1}H (1.01). *, the non-CoA structures deconjugated from the hypothetical CoA-ester intermediates were identified using UPLC–HRMS.

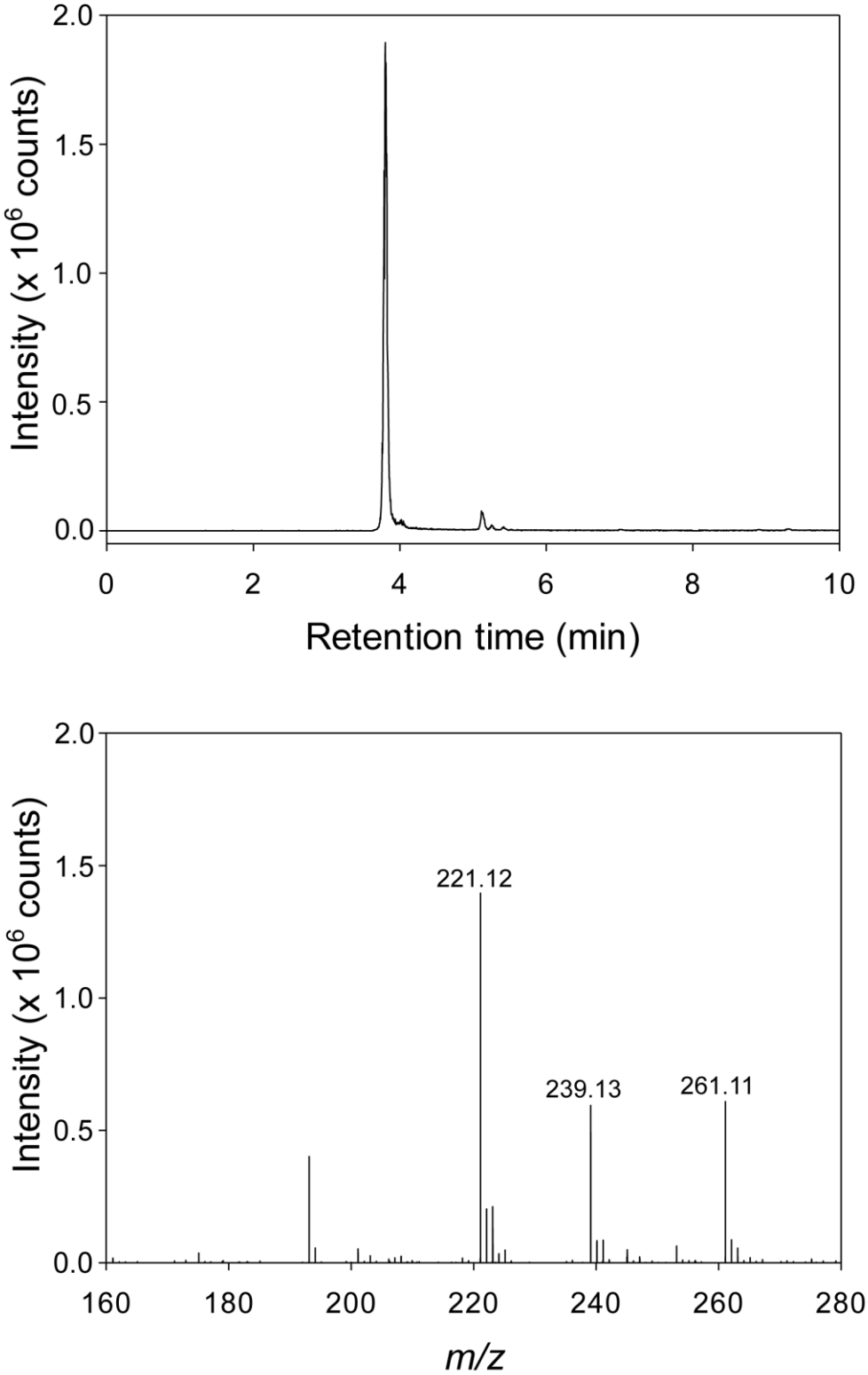

Figure S1 UPLC–ESI–HRMS analysis of the authentic standard of HIP purchased from the Sigma-Aldrich.

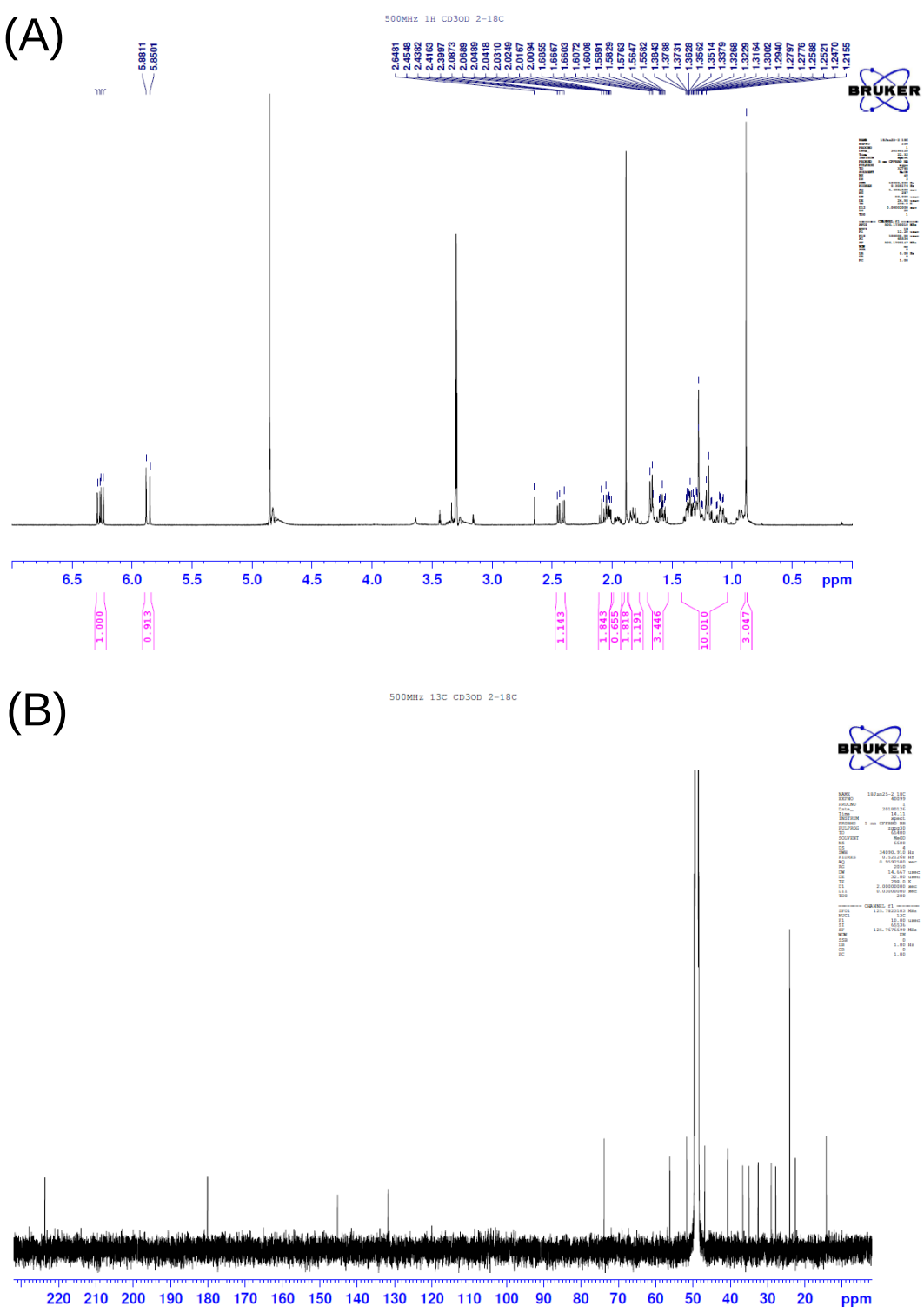

Figure S2 ^{1}H- (500 MHz) (A) and ^{13}C-NMR (125 MHz) (B) spectra of 4-norestrogenic acid.

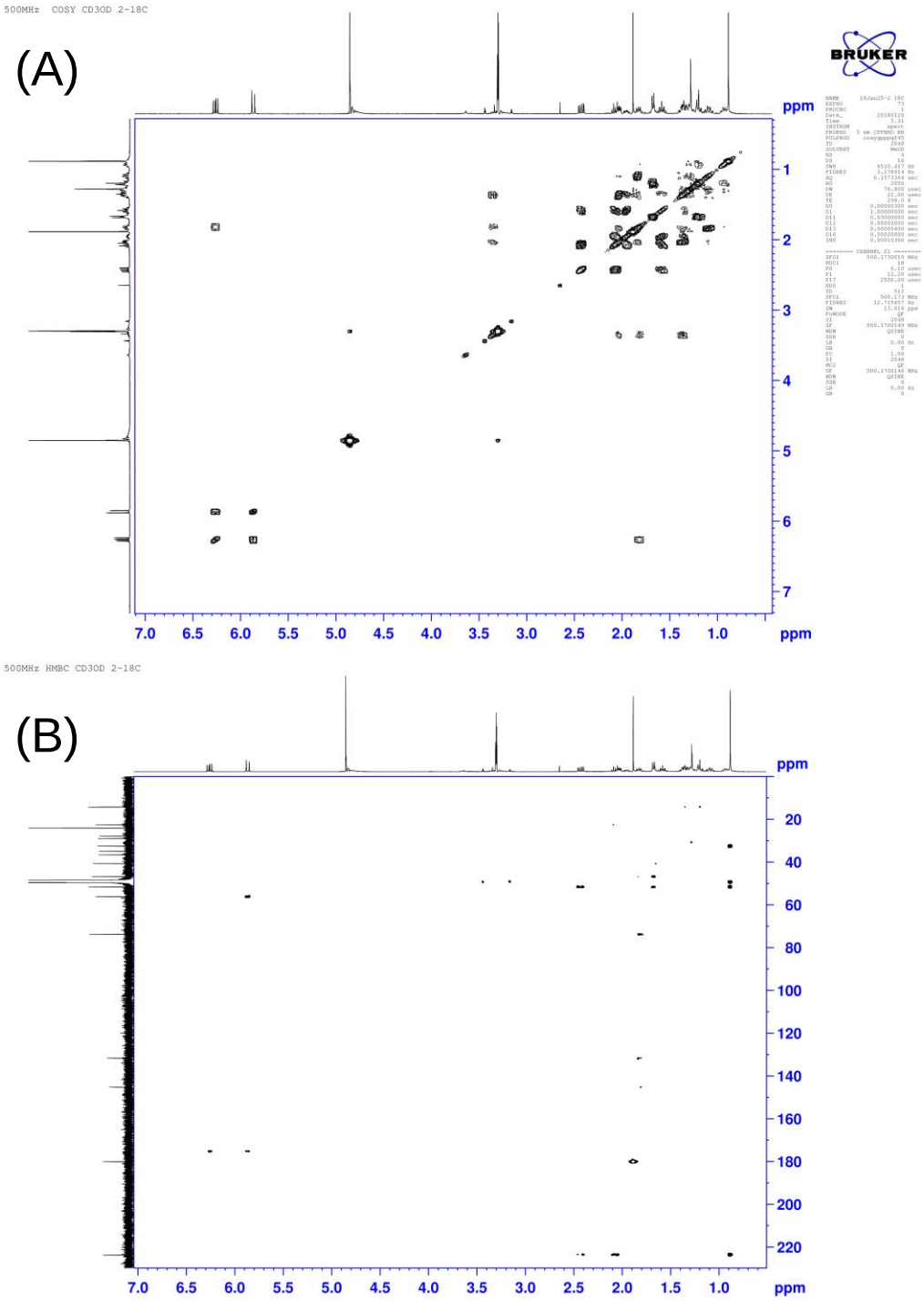

Figure S3 COSY (A) and HMBC (B) spectra of 4-norestrogenic acid.

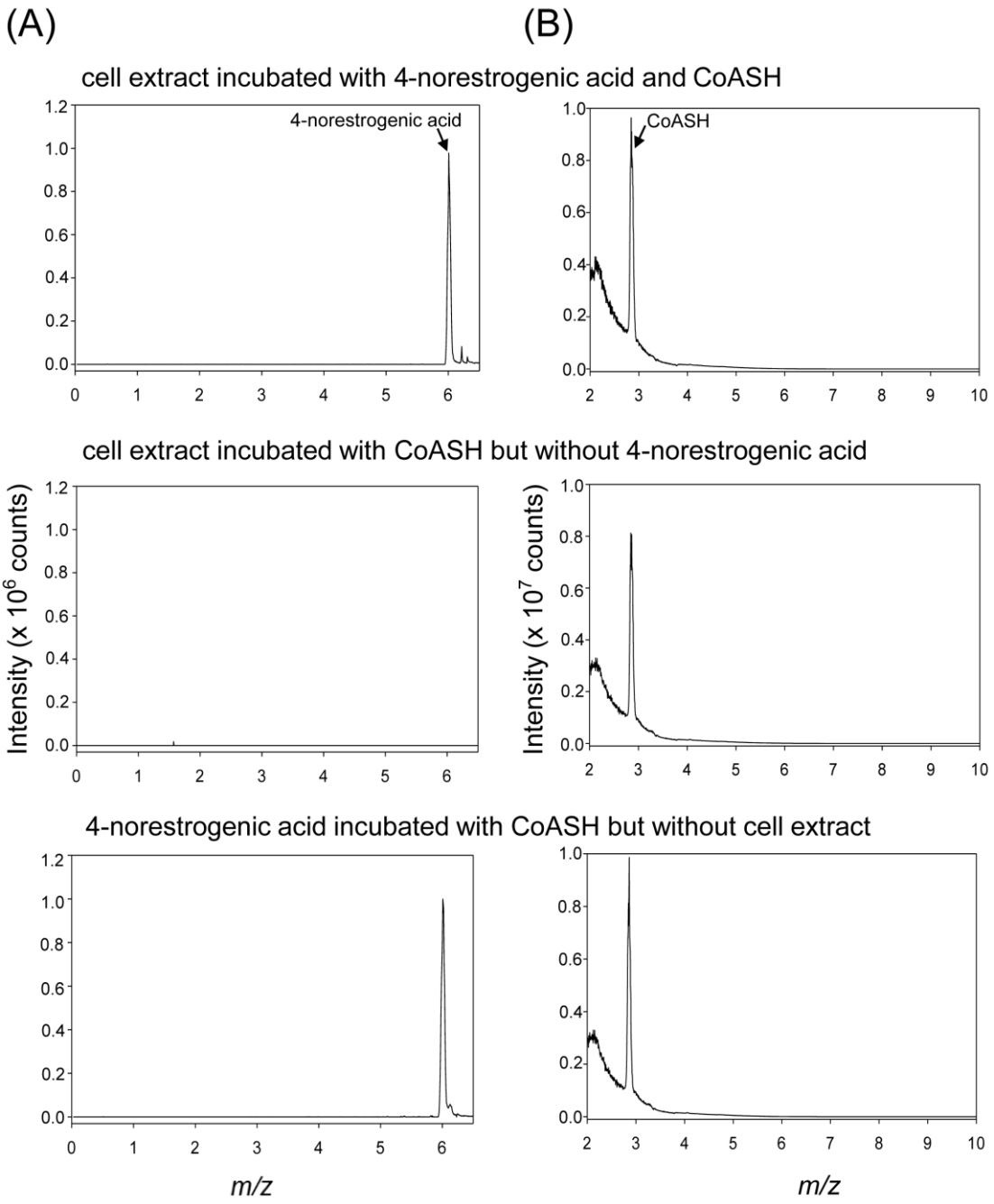

Figure S4 UPLC–ESI–HRMS analysis of the strain KC8 cell extract incubated with 4-norestrogenic acid and CoASH. (A) Extracted ion chromatograms (*m/z* = 293.17 for 4-norestrogenic acid) of the ethyl acetate extracts. (B) Total ion chromatograms (*m/z* = 700~1200) of the CoA-esters extracted from the reaction mixtures. The reaction mixture (1 mL) contained 0.5 mL of the strain KC8 proteins (20 mg/mL), 0.1 mM 4-norestrogenic acid, 1 mM CoASH, 5 mM ATP, and 10 mM $MgSO_4$. Negative controls were reaction mixtures without 4-norestrogenic acid (middle panel) or soluble proteins (lower panel). The reaction mixtures were incubated at 30 ^oC for 16 hours. The 4-norestrogenic acid and CoA-esters were extracted through liquid-liquid partition and solid phase extraction, respectively.

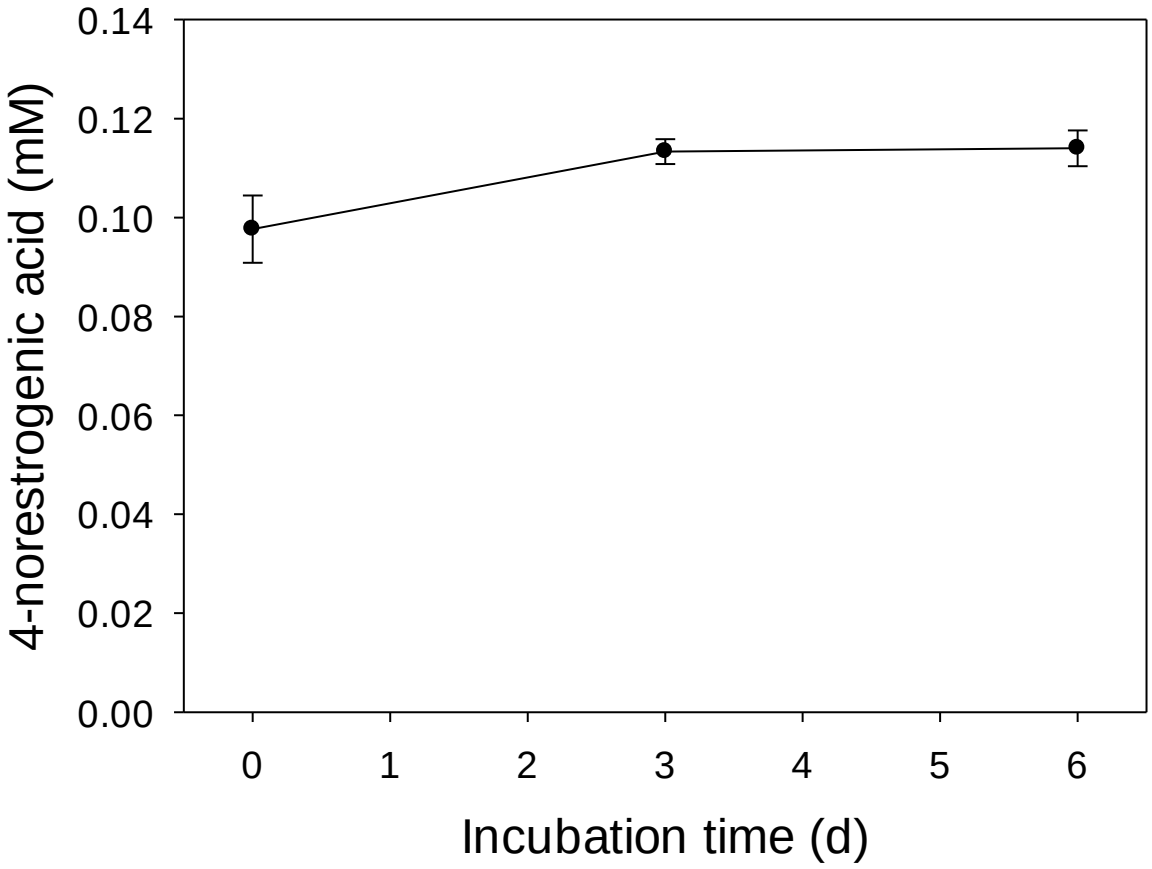

Figure S5 Incubation of strain KC8 cells with 4-norestrogenic acid (0.1 mM) for 6 days. After different time intervals of incubation, samples (0.5 mL) were withdrawn from the bacterial culture. The bacterial cells were removed through centrifugation. 4-norestrogenic acid remaining in the supernatant was extracted using ethyl acetate, and the extracted 4-norestrogenic acid was quantified through UPLC–ESI–HRMS.