Supporting Information

Wikoff *et al.* 10.1073/pnas.0812874106

SI Materials and Methods

GC/MS Profiling. We dried 8 μL of methanol extracted plasma by using a SpeedVac in a glass vial, and 75 μL of BSTFA + 1% TMCS (Pierce) was added, capped, and incubated at 37 °C for 1 h. This mixture was transferred to a GC autosampler vial with a 100-μL glass insert; 2.5 μL was injected without a split into an Agilent 6890 GC/MS instrument equipped with a HP5-MS column (J&W Scientific/Agilent) with a length of 30 m, ID of 0.25 mm, and film thickness of 0.25 μm. The injector port temperature was 290 °C and the transfer line temperature was 280 °C. The flow rate was 1.2 mL/min with a total run time of 27.5 min. The temperature program was 50 °C for 5 min, followed by a gradient of 5 °C per minute to 300 °C, followed by a hold at 300 °C for 10 min for a total run time of 55 min per sample. A 20-min blank run was included between each sample. The mass spectrometer was scanned from *m*/*z* 50 to 700.

APCI Profiling. For APCI-TOF profiling, 5 μL of methanol-extracted plasma was injected onto a SB-C18 Zorbax column (Agilent) 2.1-mm ID × 150-mm length at a flow rate of 250 μL/min. Mobile phase A was H_2O + 0.1% formic acid, and B was MeOH + 0.1% formic acid, with a gradient from 10% to 95% B from 2 to 30 min, and to 10% B from 33 to 40 min. A standard APCI source was used with a capillary voltage of 4,000 V, 35 psig nebulizer, 6.0 L/min drying gas, a vaporizer temp of 325 °C with a gas temperature of 350 °C and a corona discharge of 6 μA, and fragmentor of 125 V. The TOF was scanned from *m*/*z* 50 to 500. The injection order was alternated between the 2 groups of samples, and a 20-min gradient wash was run between each sample. Samples were profiled by using APCI positive mode with the TOF (Agilent, model 6210).

Microbial Cultures to Assess Indole-3-Propionic Acid (IPA) Production. Microbial strains were grown under anaerobic conditions according to ATCC guidelines until the culture achieved an OD_{600} of ≈1.0 or maximal density if the microbe could not achieve OD_{600} of ≈1.0 in broth culture. See Table S2 for a list of strains used in the study; 1 mL of culture was filtered with a 0.2-μm filter and stored at −80 °C. Samples were brought to room temperature, and a 50-μL aliquot was methanol extracted and prepared for LC/MS as were the serum samples. Samples were examined for detectable quantities of IPA. IPA was detected in all *Clostridium sporogenes* isolates under all growth conditions screened. *C. sporogenes* ATCC 15579 was selected for further experimentation due to the availability of its genomic sequence.

Mouse Colonization Experiments. All experiments involving live animals were approved by the institutional review board (IACUC) and conducted in an AALAS-accredited facility.

We aseptically introduced 12 female GF Swiss Webster mice age 6–8 week (Taconic) into semirigid isolators (Charles River Labs), were maintained on irradiated Pico-Vac Lab Rodent Diet (Purina), and given autoclaved water ad libitum. Nine mice were given a 100-μL suspension of *C. sporogenes* (ATCC 15579) produced from an overnight TSB culture diluted 1:100 in LB containing 40% glycerol. Two animals were removed from the isolator at 0, 5, and 10 days postinoculation, and were anesthetized with isoflurane, 50 μL of blood was collected retro-orbitally into clay capped capillary tubes, and, while under anesthesia, animals were euthanized by cervical dislocation. Also, 3 animals were treated in a similar manner 24 h postinoculation. Three animals were maintained as GF for 10 days and had blood drawn as above to serve as controls. Serum was separated from blood cells, methanol extracted, and analyzed as described previously.

IPA Clearance Studies. All experiments involving live animals were approved by the institutional review board (IACUC) and conducted in an AALAS-accredited facility.

We ordered 20 GF Swiss Webster female mice aged 6–8 weeks from Taconic Farms. Animals were removed from the GF shipper 24 h after arrival and placed in a biosafety cabinet that had been decontaminated with 3% Nolvasan. The mice were randomly divided into four groups (n = 5 animals per group) and administered 10, 20, 40 mg/kg IPA (Acros Organics) or sterile PBS vehicle by i.p. injection. Animals were anesthetized with isoflurane (Henry Schein), and had 50 μL of blood drawn retro-orbitally at 0, 2, and 6 h postinjection. Serum was separated from blood cells by centrifugation of capillary tubes for 2 min in a BD Adams Autocrit Ultra 3 Centrifuge, tubes were broken above the blood cell-serum interface, and the serum layer was collected into capped tubes. The serum was then methanol extracted and analyzed for the presence of IPA. All experiments involving live animals were approved by the institutional review board (IACUC) and conducted in an AALAS-accredited facility.

IPA Quantitation in Mouse Serum. Samples were prepared as described above. LC/MS data were recorded by using an Agilent 6520 Accurate-Mass Q-TOF LC/MS system with dual ESI source and an Agilent 1200 HPLC system; 10 μL was injected onto column (Agilent Zorbax 300SB-C18, 5 μm, 150 × 2.0 mm). Eluent A was 0.1% (vol/vol) formic acid in water; eluent B was 0.1% (vol/vol) formic acid in acetonitrile. The gradient consisted of 5% B for 5 min, followed by a gradient to 95% B >45 min, hold at 95% B for 5 min, and a reequilibration at 5% B for 10 min. The flow rate was 200 μL/min. The mass spectrometric data were collected in profile mode by using − ESI. The gas temperature was 350 °C. The drying gas flow rate was 5 L/min, and the pressure of the nitrogen nebulizer gas was 30 psig. The capillary, fragmentor, skimmer, and OCT1 RF Vpp voltages were 3,500 V (−), 150 V, 65 V, and 750 V, respectively. A calibration curve was made by running IPA standard solutions of 50, 200, 500, and 1,000 ng/mL.

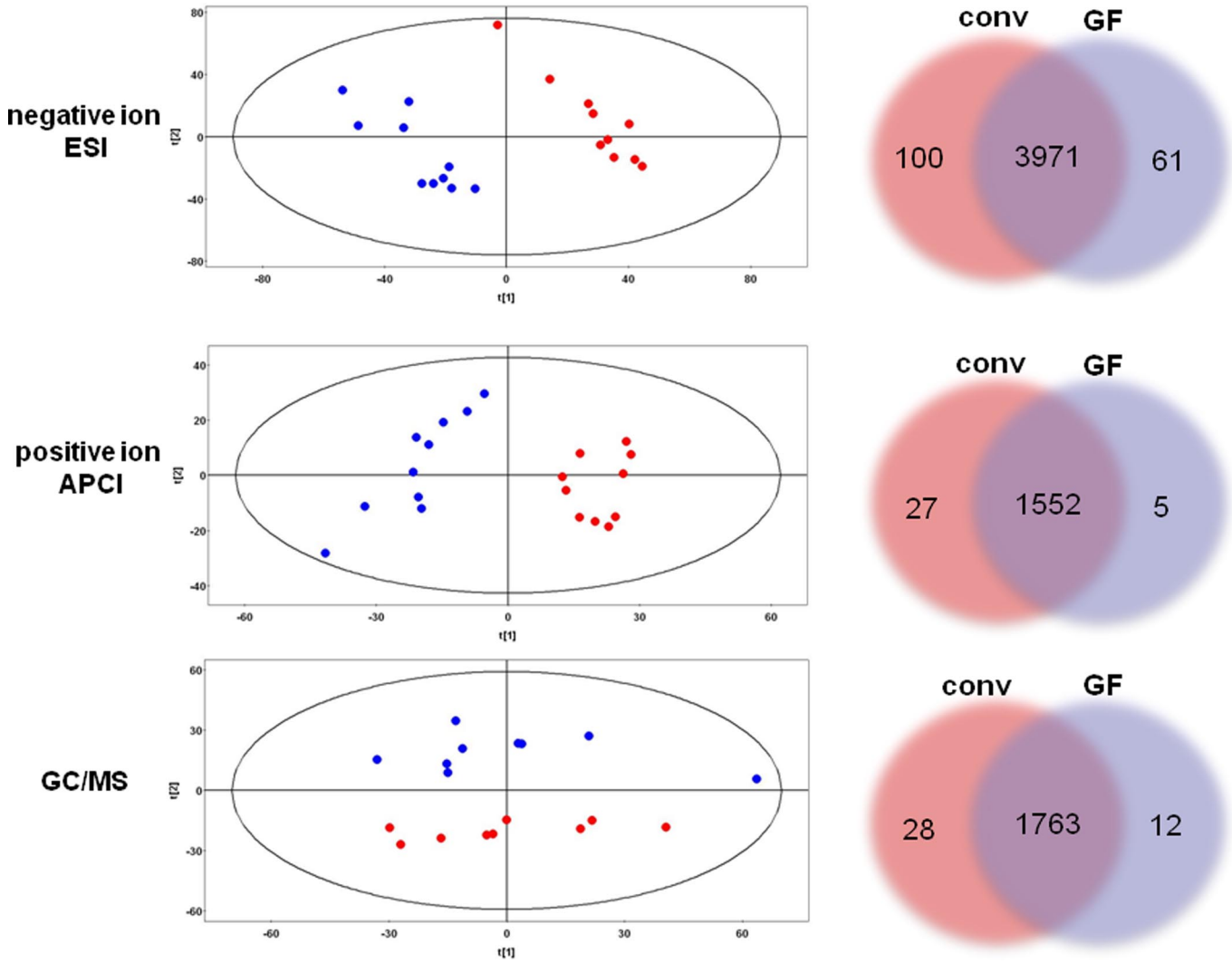

Fig. S1. PCA analysis of the profiling data and Venn diagrams for the individual features observed obtained employing − ESI (*Top*), + APCI (*Middle*), and GC/MS (*Bottom*).

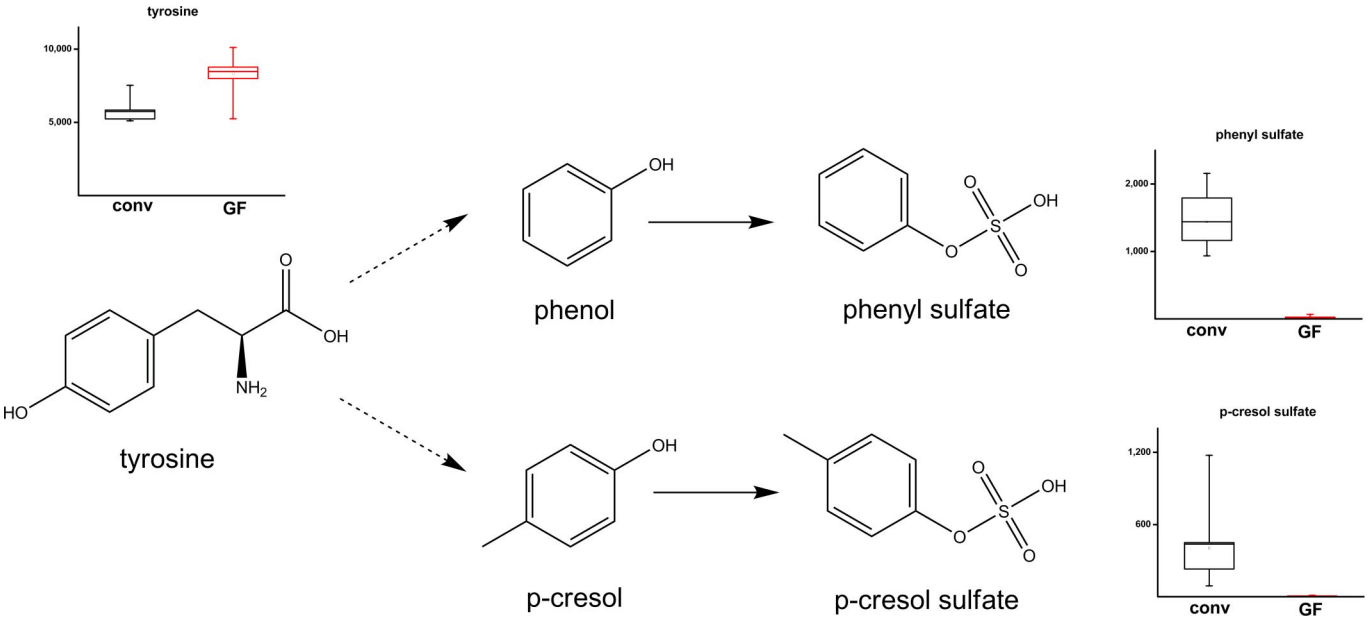

Fig. S2. Differences in metabolites that arise due to the action of the microbiome on tyrosine metabolism. The integrated signal intensities plotted on the *y* axes are reduced by a factor of 1,000.

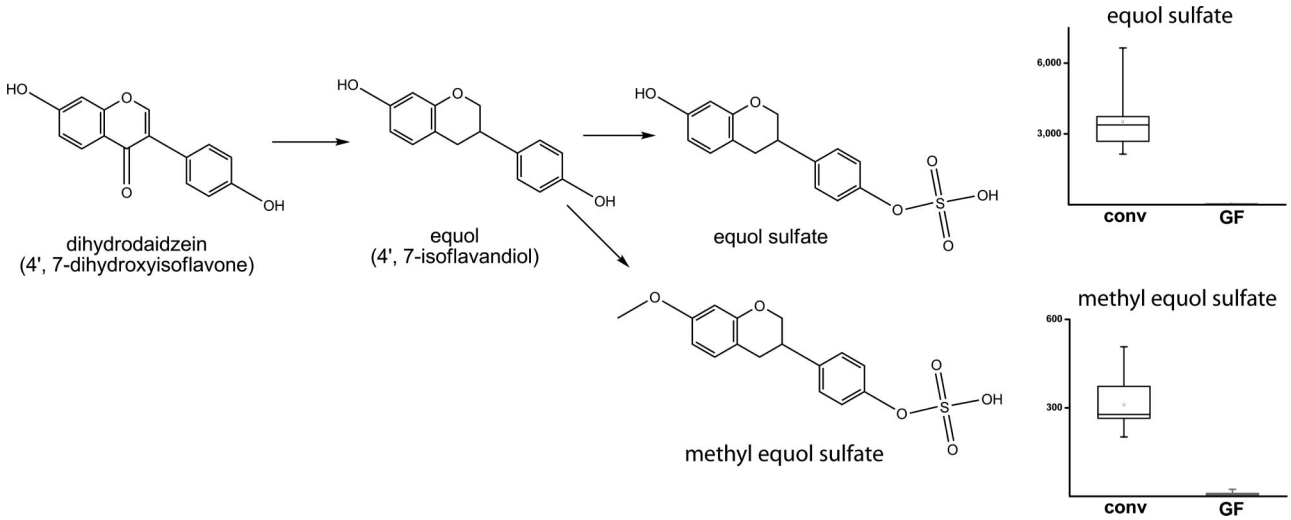

Fig. S3. Differences in metabolites that arise due to the action of the microbiome on the dietary isoflavone dihydrodaidzein. The integrated signal intensities plotted on the *y* axes are reduced by a factor of 1,000.

Table S1. Summary of mass spectrometric measurements based on which molecular identifications were made

Compound ID	Molecular formula	Mode observed	M	*m/z* obs	*m/z* calc, neutral	ppm error	RT, min	CE	Major fragments, QTOF
Indole compounds									
Tryptophan	$C_{11}H_{12}N_2O_2$	ESI +, ESI −, AP +, GCMS	+	205.0958	204.0898	−9.28	7.8	35	118.069, 91.058, 143.077, 130.07
N-acetyltryptophan	$C_{13}H_{14}N_2O_3$	ESI +	+	247.1061	246.1004	−8.77	18.7	25	159.096, 146.064, 132.083, 170.064, 188.076, 118.069
Indoxyl sulfate (indican)	$C_8H_7NO_4S$	ESI −	−	212.0032	213.0095	—	5.5	—	+132.044, 79.959
Serotonin	$C_{10}H_{12}N_2O$	ESI +, AP +	+	177.1008	176.0949	−11.22	5.1	35	115.054, 159.068, 133.053, 105.068, 77.039
Indole-3-propionic acid	$C_{11}H_{11}NO_2$	ESI +, ESI −, AP +	+	190.0851	189.0789	−8.96	23.1	—	130.064, 103.054, 77.039, 55.018
Phenyl compounds									
Phenylalanine	$C_9H_{11}NO_2$	ESI +, ESI −, AP +	+	166.0849	165.0789	−11.46	4.8	—	120.0801, 103.0540, 91.0544, 77.0388
Tyrosine	$C_9H_{11}NO_3$	AP +, ESI +	+	182.0804	181.0739	−7.24	2.2	35	119.0500, 107.0502, 95.0501, 91.0556, 77.0389, 65.0386, 56.9438
Hippuric acid	$C_9H_9NO_3$	ESI −, AP +	−	178.0515	179.0582	6.08	4.5	10	134.061, 77.041
Phenylacetylglycine	$C_{10}H_{11}NO_3$	ESI +, AP +	−	194.0795	193.0738	−11.43	12.1	—	91.058, 65.042
Phenyl sulfate	$C_6H_6O_4S$	ESI −	−	172.9920	173.9986	6.62	5.5	—	+93.035, 79.957
p-Cresol sulfate	$C_7H_8O_4S$	ESI −	−	187.0080	188.0143	7.99	8.3	—	+80, 107
Phenylpropionylglycine	$C_{11}H_{13}NO_3$	ESI +	+	208.0952	207.0895	−10.4	17.4	35	—
Cinnamoylglycine	$C_{11}H_{11}NO_3$	ESI +, ESI −	+	206.0802	205.0738	−7.37	19.2	35	103.0542, 77.0394, 131.04803
Others									
Urate	$C_5H_4N_4O_3$	ESI −, AP +	−	167.0215	168.0283	5.90	3.7	25	69.0116, 124.0166, 96.0203
Creatinine	$C_4H_7N_3O$	AP +	+	114.0655	113.0589	−10.8	1.4	—	—
Equol sulfate	$C_{15}H_{14}SO_6$	ESI −	−	321.0426	322.0511	−2.13	21	25	+241.0875, 147.0442, 135.0447, 121.0302, 119.0504, 79.9582
Methyl equol sulfate	$C_{16}H_{16}SO_6$	ESI −	−	335.0621	336.0667	9.44	25.2	25	+255.1032, 121.0309, 79.9587, 59.0099

M refers to the ESI ionization mode used for peak integration and compound identification. RT, retention time. CE refers to the collision energy employed to obtain the tandem MS spectra. All compounds identified as sulfates were also verified to have an isotope pattern for which the M + 2 peak was higher in intensity than expected compared with an isobaric species not containing sulfur.

Table S2. Individual bacterial species and their culture conditions that were assayed for the production of IPA

Division	Strain	ATCC number(s)	Growth medium
Actinobacteria	*Actinomyces odontolyticus* Batty	17982	BBL Actinomyces broth
Actinobacteria	*Collinsella aerofaciens* Eggerth	25986	ATCC medium 1053
Archaea	*Methanobrevibacter smithii*	35061	ATCC medium 1340
Bacteroidetes	*Alistipes putredinis*	29800	ATCC medium 1490
Bacteroidetes	*Bacteroides caccae*	43185	BBL cooked meat medium
Bacteroidetes	*Bacteroides capillosus*	29799	BBL cooked meat medium
Bacteroidetes	*Bacteroides ovatus*	8483	BBL cooked meat medium
Bacteroidetes	*Bacteroides stercoris*	43183	BBL cooked meat medium
Bacteroidetes	*Bacteroides thetaiotaomicron*	29148	BBL cooked meat medium
Bacteroidetes	*Bacteroides uniformis*	8492	BBL cooked meat medium
Bacteroidetes	*Parabacteroides merdae*	43184	BBL cooked meat medium
Firmicutes	*Clostridium leptum*	29065	ATCC medium 1490
Firmicutes	*Clostridium ramosum*	25582	ATCC medium 38
Firmicutes	*Clostridium scindens*	35704	ATCC medium 1053
Firmicutes	*Clostridium sporogenes*	17888, 319, 15579	TSB broth, BBL cooked meat medium*
Firmicutes	*Clostridium symbiosum*	14940	ATCC medium 1490
Firmicutes	*Coprococcus eutactus*	27759	ATCC medium 735
Firmicutes	*Eubacterium dolichum*	29143	ATCC medium 593
Firmicutes	*Eubacterium ventriosum* Tissier	27560	ATCC medium 593
Firmicutes	*Micromonas micros*	33270	ATCC medium 1102
Firmicutes	*Ruminococcus gnavus*	29149	ATCC medium 1490
Firmicutes	*Ruminococcus obeum*	29174	ATCC medium 1490
Firmicutes	*Ruminococcus torques*	27756	ATCC medium 735
Proteobacteria	*Escherichia coli*	700926, 700891	TSB broth

*ATCC number 15579 was tested in both media and was able to grow and produce IPA in both media

Table S3. Serum concentration of IPA measured in plasma samples after intraperitoneal injection of IPA

Treatment	Animal no.	IPA post-injection conc., μg/mL		
		0 h	1 h	6 h
Vehicle	1	0.00	0.00	0.08
	2	0.00	0.00	0.00
	3	0.00	0.00	0.00
	4	0.00	0.00	0.00
	5	0.00	0.00	0.00
	Vehicle mean	0.00	0.00	0.02
	Vehicle SD	0.00	0.00	0.04
10 mg/kg IPA	1	0.00	18.24	0.78
	2	0.00	22.76	0.79
	3	0.00	20.70	0.89
	4	0.00	26.70	—
	5	0.00	—	—
	10 mg/kg mean	0.00	22.10	0.82
	10 mg/kg SD	0.00	3.58	0.06
20 mg/kg IPA	1	0.00	31.09	0.53
	2	0.00	33.24	1.42
	3	0.00	57.06	1.66
	4	0.00	31.16	0.09
	5	0.00	24.25	1.11
	20 mg/kg mean	0.00	35.36	0.96
	20 mg/kg SD	0.00	12.60	0.64
40 mg/kg IPA	1	0.00	35.43	0.91
	2	0.06	—	—
	3	0.00	53.81	3.87
	4	0.00	33.22	2.49
	5	1.60	55.03	4.31
	40 mg/kg mean	0.33	44.37	2.90
	40 mg/kg SD	0.71	11.65	1.53

All values are in μg/mL of serum, and the limit of detection was 0.053 μg/mL.