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Supplementary Materials for

Bioactive polyamine production by a novel hybrid system comprising multiple indigenous gut bacterial strategies

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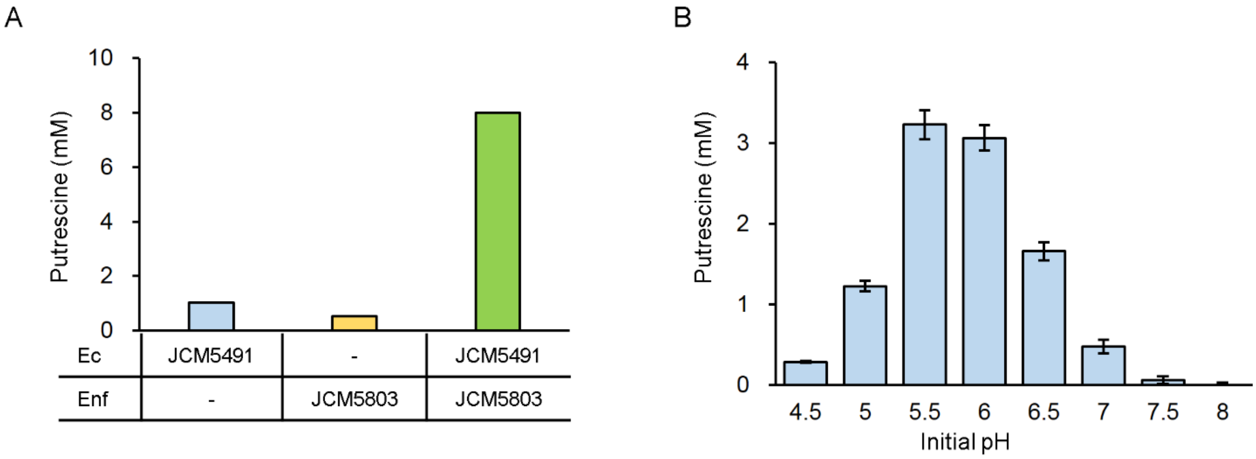

Fig. S1. Induction of putrescine production by coculture of *E. coli* and *En. faecalis*. (**A**) Extracellular putrescine concentration in monocultures of *E. coli* JCM5491 and *En. faecalis* JCM 5803^T, and a coculture of both strains. Error bars represent standard errors (SEs). Ec: *E. coli*, Enf: *En. faecalis*. (**B**) Effect of pH on extracellular putrescine concentration in cocultures of *E. coli* and *En. faecalis*.

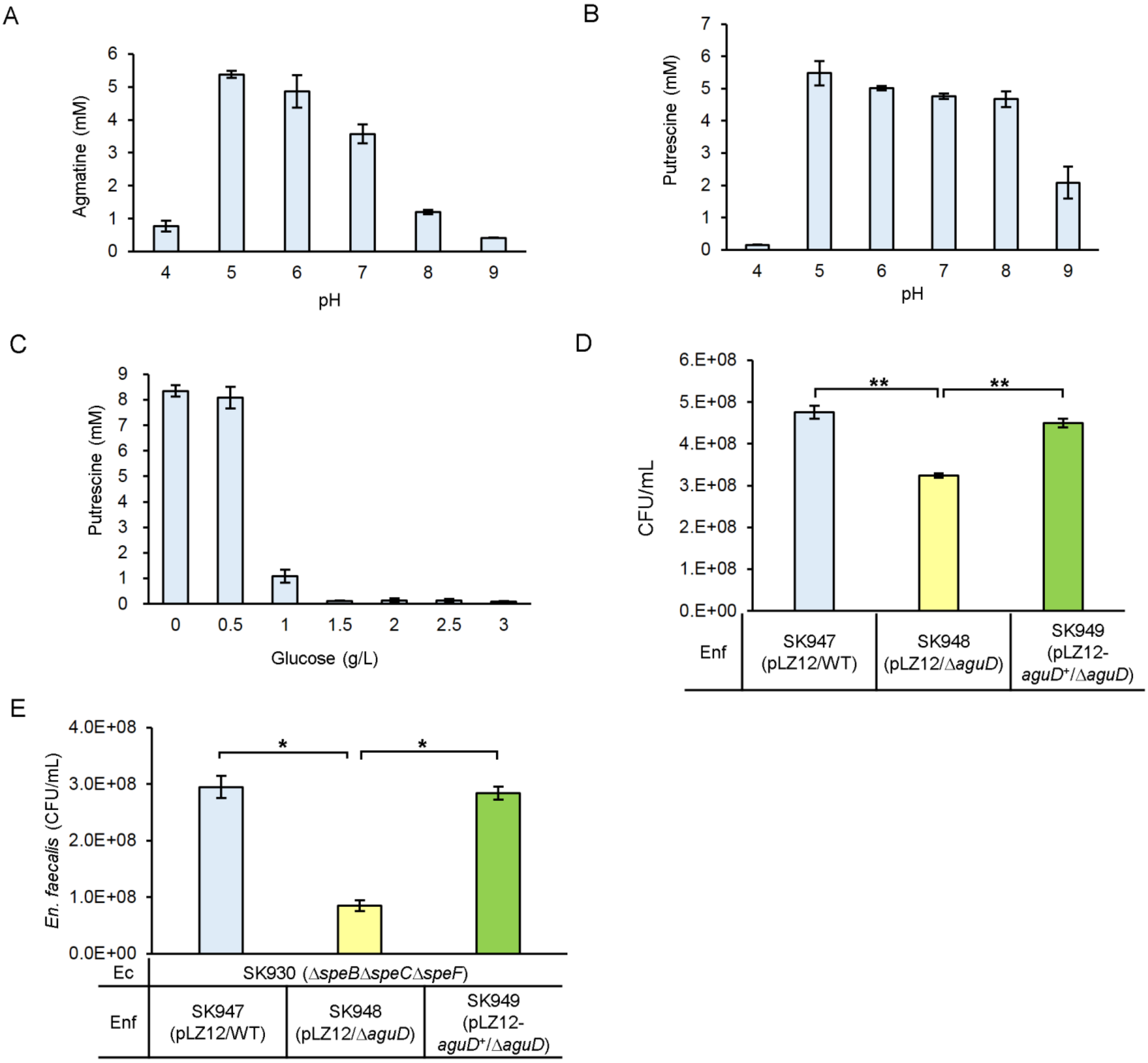

Fig. S2. Effects of extracellular pH and glucose concentration on bacterial metabolism in monocultures of *En. faecalis* and *E. coli*
(**A**) Extracellular agmatine concentration in monocultures of wild-type *E. coli* (MG1655) in LB-RGC at different pH. (**B**) Extracellular putrescine concentrations in monocultures of wild-type *En. faecalis* (V583) cultured in medium at different pH values containing 5 mM agmatine. (**C**) Extracellular putrescine concentration in a monoculture of wild-type *En. faecalis* (V583) in medium containing 5 mM agmatine and different concentrations of glucose. (**D**) Viable cell count of wild-type *En. faecalis* (SK947), *aguD*-deleted *En. faecalis* (SK948), or *aguD*-complemented *En. faecalis* (SK949) monocultured in medium containing 5 mM agmatine at pH 6. (**E**) Viable cell counts of *En. faecalis* when putrescine-deficient *E. coli* (SK930) was cocultured with wild-type *En. faecalis* (SK947), *aguD*-deleted *En. faecalis* (SK948), or *aguD*-complemented *En. faecalis* (SK949) in LB-RGC medium. Mono- and cocultures of these bacteria were conducted under anaerobic conditions at 37ºC for 24 h.
Error bars represent SEs. Student's *t*-tests; **p* < 0.05, ***p* < 0.01.
Enf: *En. faecalis*

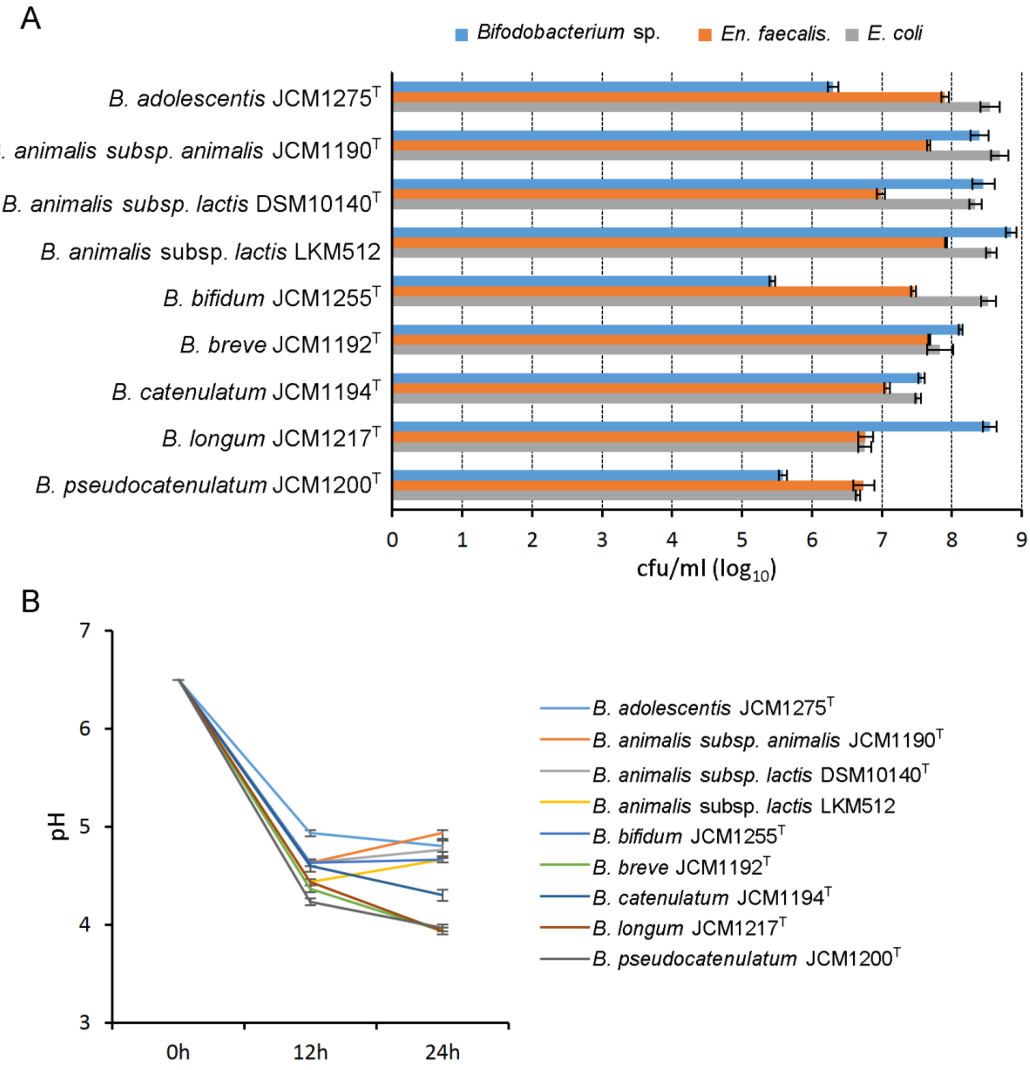

Fig. S3. Viable bacterial counts and change of extracellular pH in cocultures of putrescine-deficient *E. coli* (SK930), wild-type *En. faecalis* (V583), and each *Bifodobacterium* sp.

(**A**) Viable bacterial counts. Coculture of these bacteria was conducted under anaerobic conditions at 37 ºC for 24 h in LB medium containing 5 mM L-arginine, 1.5 g/L D-glucose, 5 g/L galacto-oligosaccharide, 2 mM $MgSO_4$, 60 mM NH_4Cl, and 0.5 g/L L-cysteine-hydrochloride (pH 6.5). (**B**) Change of extracellular pH.

The cultures of all strains reached pH 5.5, at which point, maximal putrescine production occurred within 12 hours. However, there was no relationship between the viable count of *Bifidobacterium* sp. and pH. Interestingly, we found that the extracellular putrescine concentration positively correlated with the number of both *E. coli* and *En. faecalis*. Specifically, in the culture with *B. animalis* subsp. *lactis* LKM512 or *B. adolescentis* JCM1275, which yielded higher putrescine production, larger numbers of *E. coli* ($10^{8.5}$ cfu/ml or more) and *En. faecalis* ($10^{7.9}$ cfu/ml or more) were detected, whereas in the culture with *B. longum* JCM1217 or *B. pseudocatenulatum* JCM1200, which induced only slight putrescine production, 10-times lower number of *E. coli* and *En. faecalis* (less than $10^{6.8}$ cfu/ml) were detected.

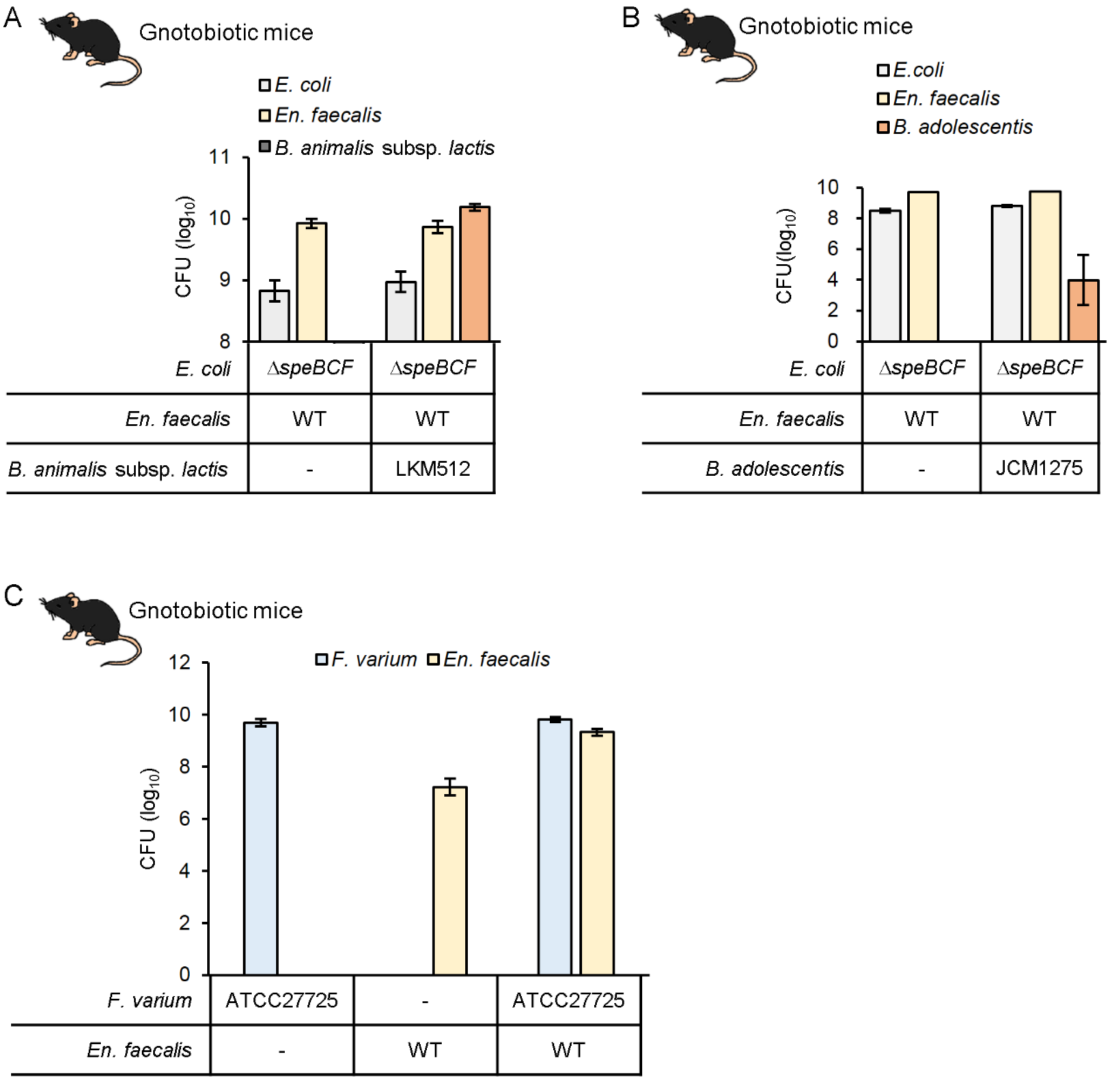

Fig. S4. Viable bacterial counts in faeces of gnotobiotic mice
(**A**) Viable bacterial counts in feces derived from gnotobiotic mice colonized with putrescine-deficient *E. coli* (SK930), wild-type *En. faecalis* (V583), and *B. animalis* subsp. *lactis* LKM512. (**B**) Viable bacterial counts in feces derived from gnotobiotic mice colonized with putrescine-deficient *E. coli* (SK930), wild-type *En. faecalis* (V583), and *B. adolescentis* JCM1275^T. (**C**) Viable bacterial counts in feces derived from gnotobiotic mice inoculated with *F. varium* ATCC 27725 and wild-type *En. faecalis* (V583). Error bars represent SEs.

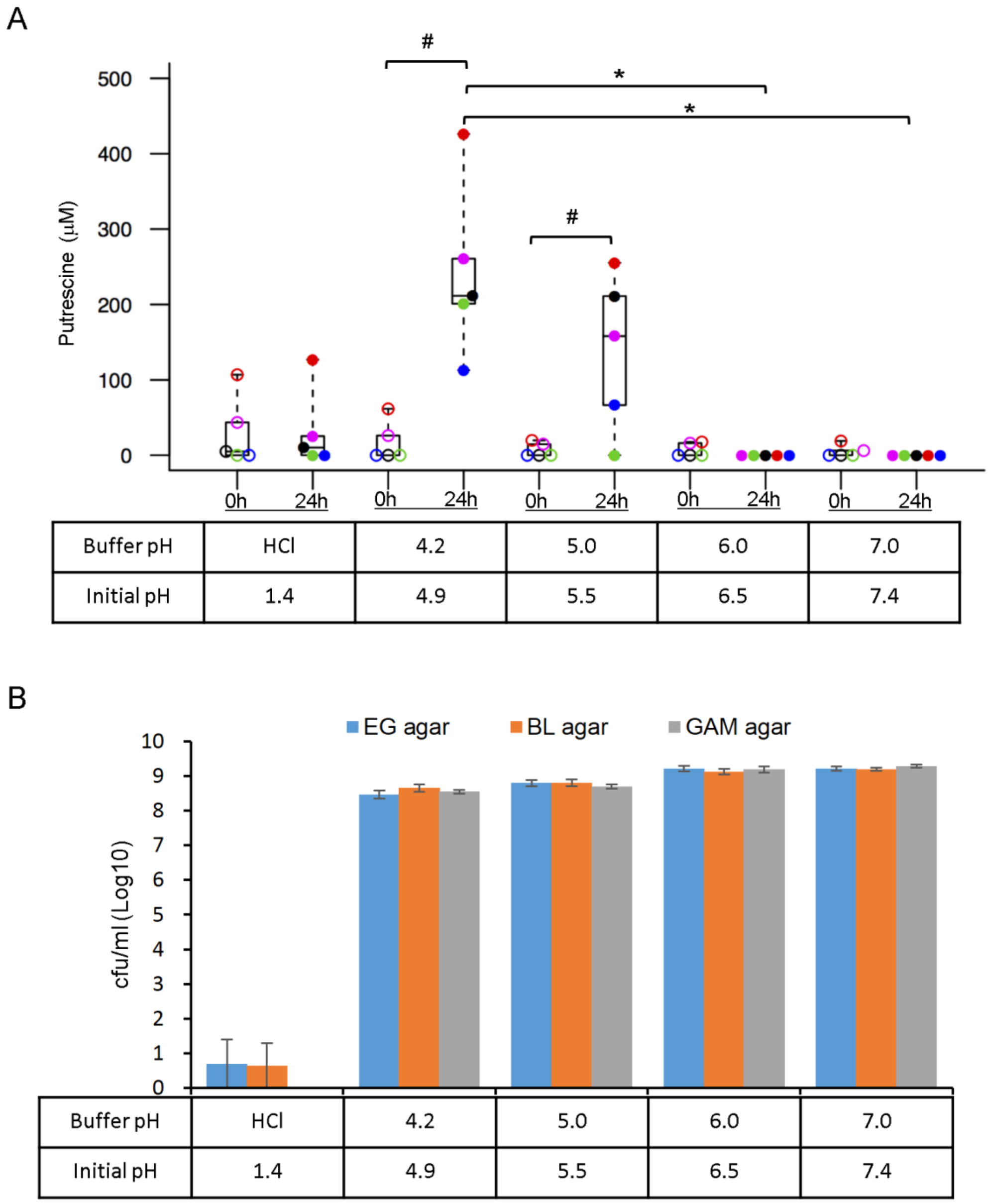

Fig. S5. Extracellular putrescine concentration and total viable bacterial counts in human feces incubated at different pH values (n = 5)

(**A**) Extracellular putrescine concentration. Same color plots indicate the same individual. Open and closed symbols indicate the putrescine concentration before and after incubation, respectively. (**B**) Total bacterial counts at 24 h were measured by cultivation on blood-liver (BL) agar plate, Eggerth-Gagnon (EG) agar plate, and GAM agar plate. Steel-Dwass test; $^*p < 0.05$, Wilcoxon signed rank test; $^{\#}p < 0.05$.

Five minutes after suspension (0 h of incubation), putrescine concentration was higher at pH 1.4 (31 µM) and pH 4.9 (18 µM) than that at pH 5.5, 6.5, and 7.4 (approximately 6 µM), indicating that bacterial cells were ruptured and a small amount of putrescine was released into the suspension at pH 1.4 and pH 4.9. Furthermore, after incubation for 24 h, at pH 4.2, putrescine concentration increased to 12-times the concentration (250 µM) observed before incubation, and the increase was observed in all samples, confirming that putrescine was produced by intestinal bacteria. In contrast, putrescine concentration was not increased at pH 1.4 because almost all bacteria were dead.

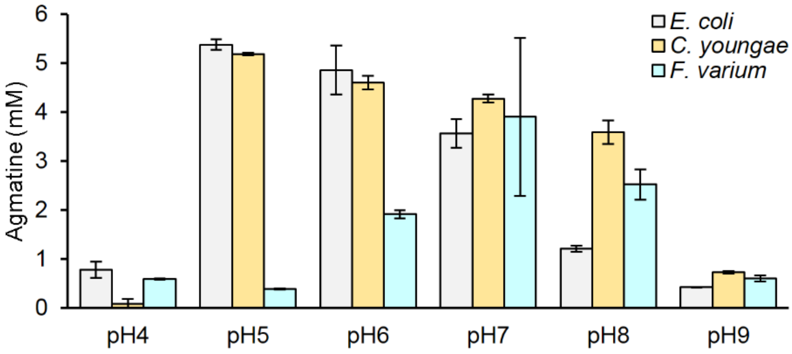

Fig. S6. Effect of pH on extracellular agmatine concentration in monocultures of *E. coli*, *C. youngae*, and *F. varium*.

Monoculture of these bacteria was conducted under anaerobic conditions at 37 ºC for 24 h in LB-RGC (pH 4.0–9.0). *C. youngae* produced agmatine due to pH decrease similar to *E. coli*, indicating the presence of bacteria possessing the AdiC/AdiA pathway, which is triggered by environmental acidification in the gut, whereas *F. varium* did not show the same trend, and the highest agmatine production was observed at neutral pH (pH 7). These findings indicate that agmatine production does not depend on environmental acidification

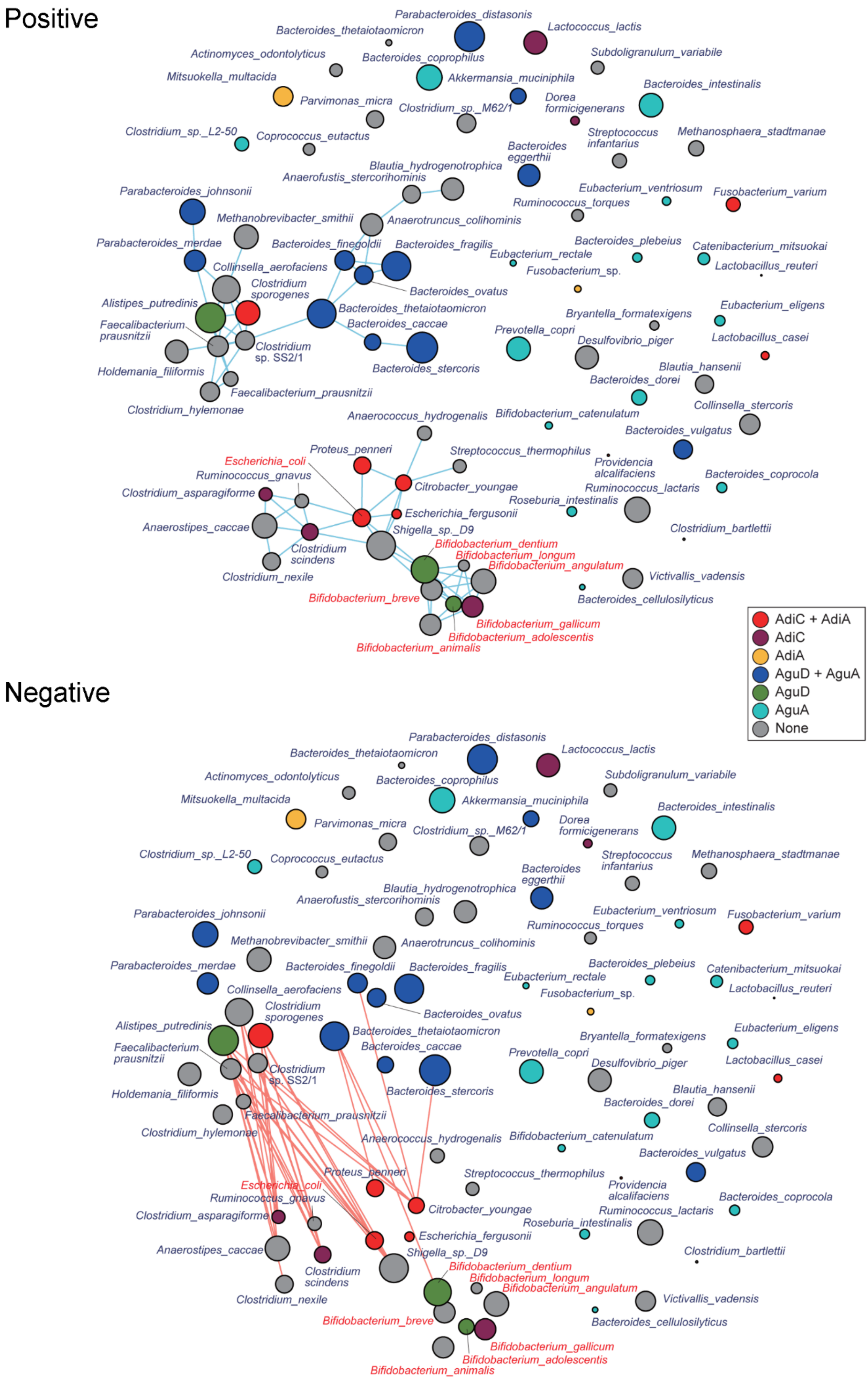

Fig. S7. Symbiont–symbiont co-occurrence networks of key genes in the putrescine production pathway using previously described human microbiome data from US metropolitan areas .
Details are shown in Fig. 5C

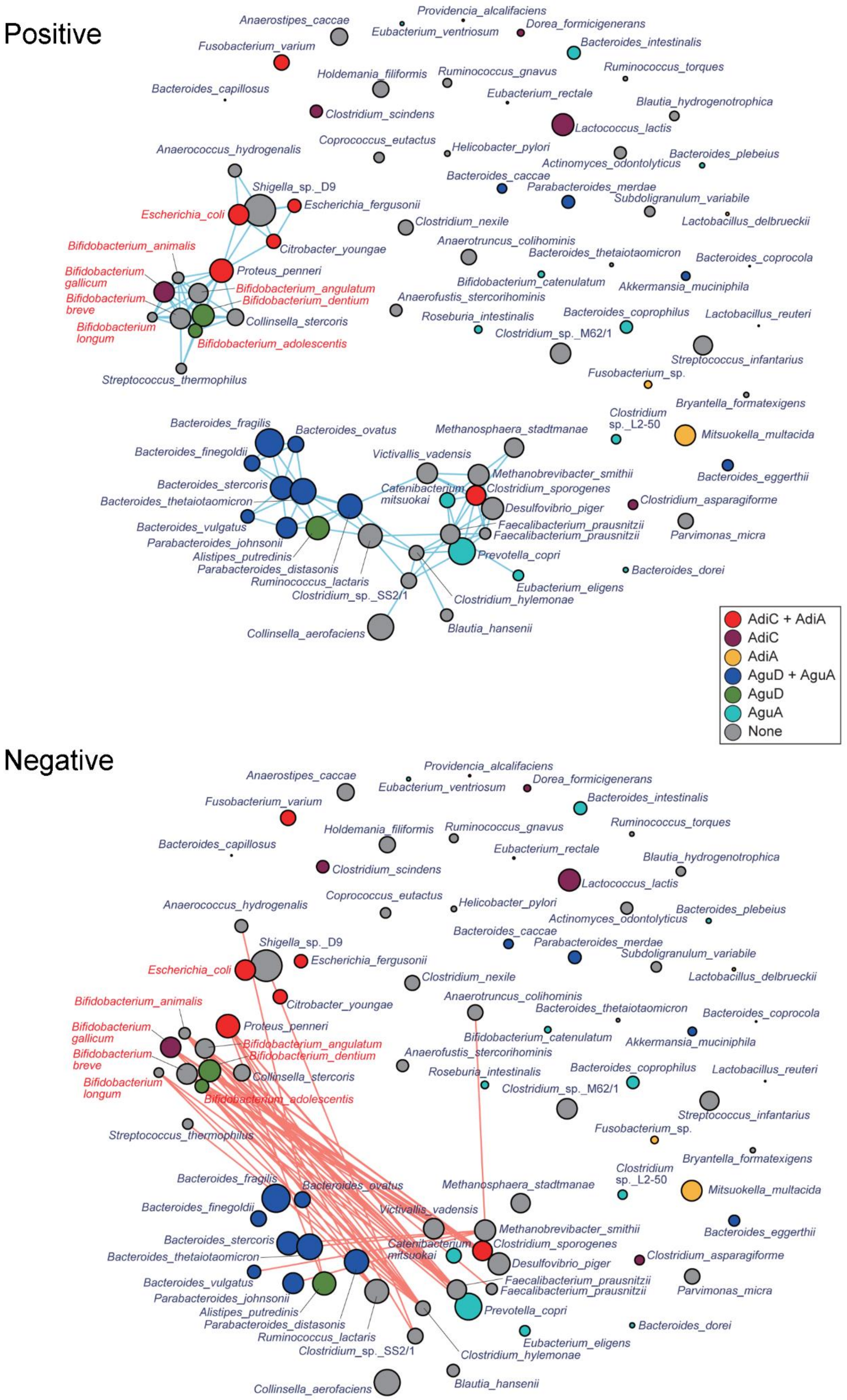

Fig. S8. Symbiont–symbiont co-occurrence network patterns of key genes in the putrescine production pathway using previously described human microbiome data from Venezuela. Details are shown in Fig. 5C

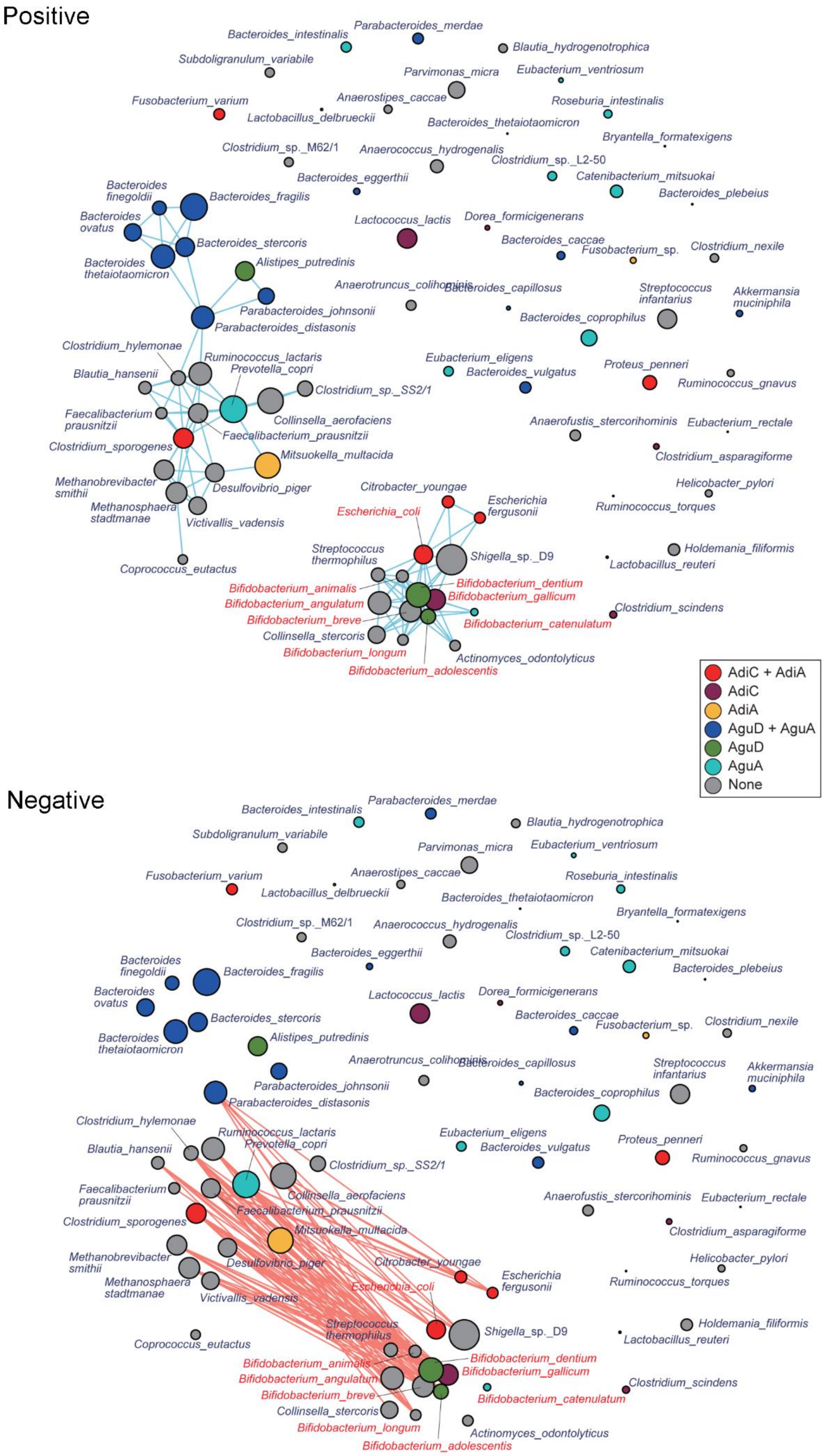

Fig. S9. Symbiont–symbiont co-occurrence network patterns of key genes in the putrescine production pathway using previously described human microbiome data from Malawi. Details are shown in Fig. 5C.

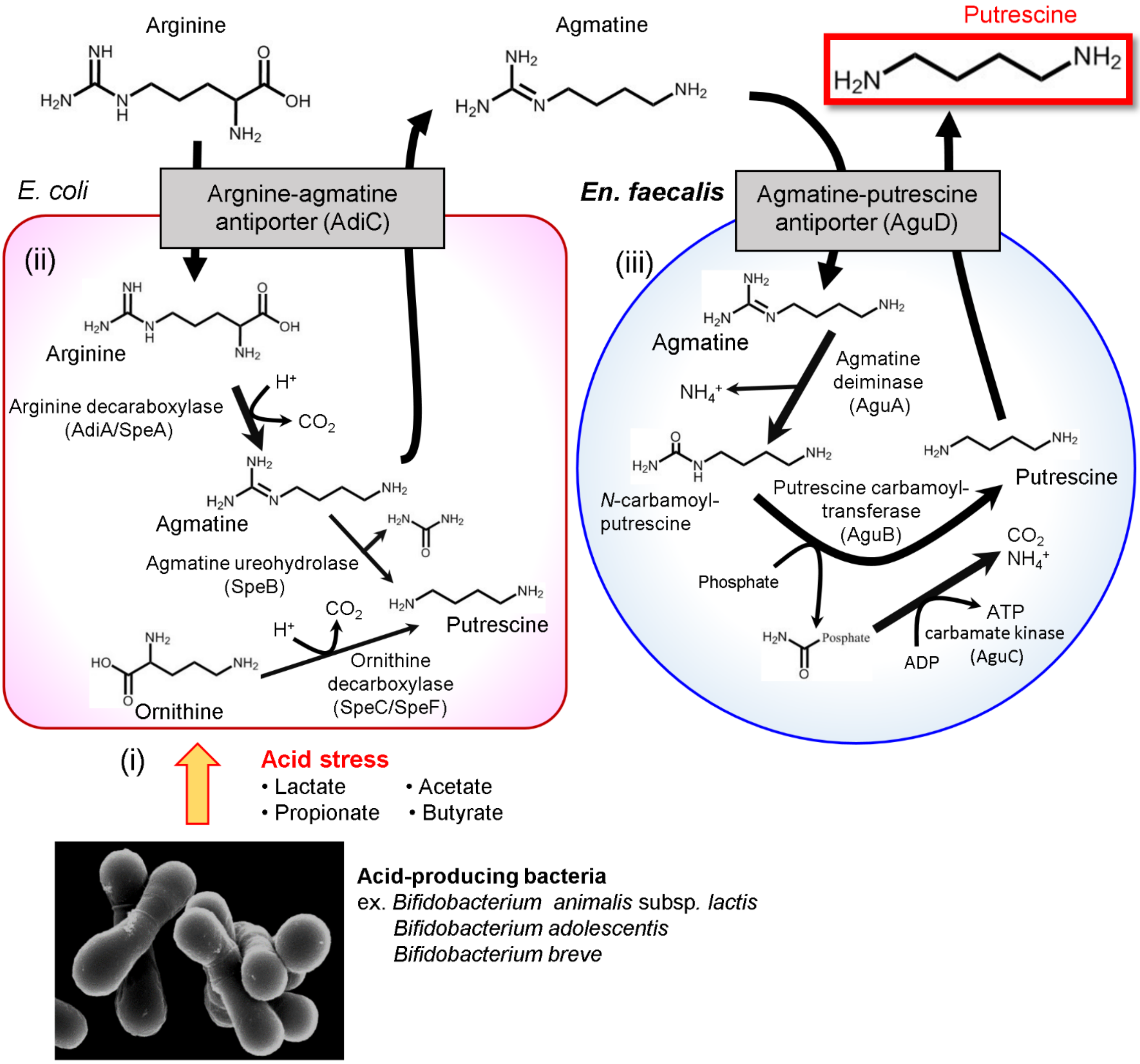

Fig. S10. Mechanistic model of a novel pathway for putrescine production from arginine through agmatine via the collaboration of three different bacterial species

(i) This pathway is triggered by environmental acidification due to acetate and lactate produced by acid-producing bacteria, represented by *Bifidobacterium* spp. (ii) In the second step, the acid-tolerance system of *E. coli* (arginine-dependent acid resistance system) is activated by acidic stress to exchange agmatine inside the cell for arginine present outside. (iii) In the third step, the energy production system of *En. faecalis* (agmatine deiminase system) is activated to exchange putrescine inside the cell for agmatine present outside (owing to the acid-tolerance system of *E. coli*). Putrescine is a byproduct of the collaboration between these different bacterial species

Abbreviations: Arg, arginine; Agm, agmatine; Put, putrescine; AdiA, arginine decarboxylase; AdiC, arginine/agmatine antiporter; AguA, agmatine deiminase; AguB, putrescine carbamoyltransferase; AguC, carbamate kinase; AguD, agmatine/putrescine antiporter.

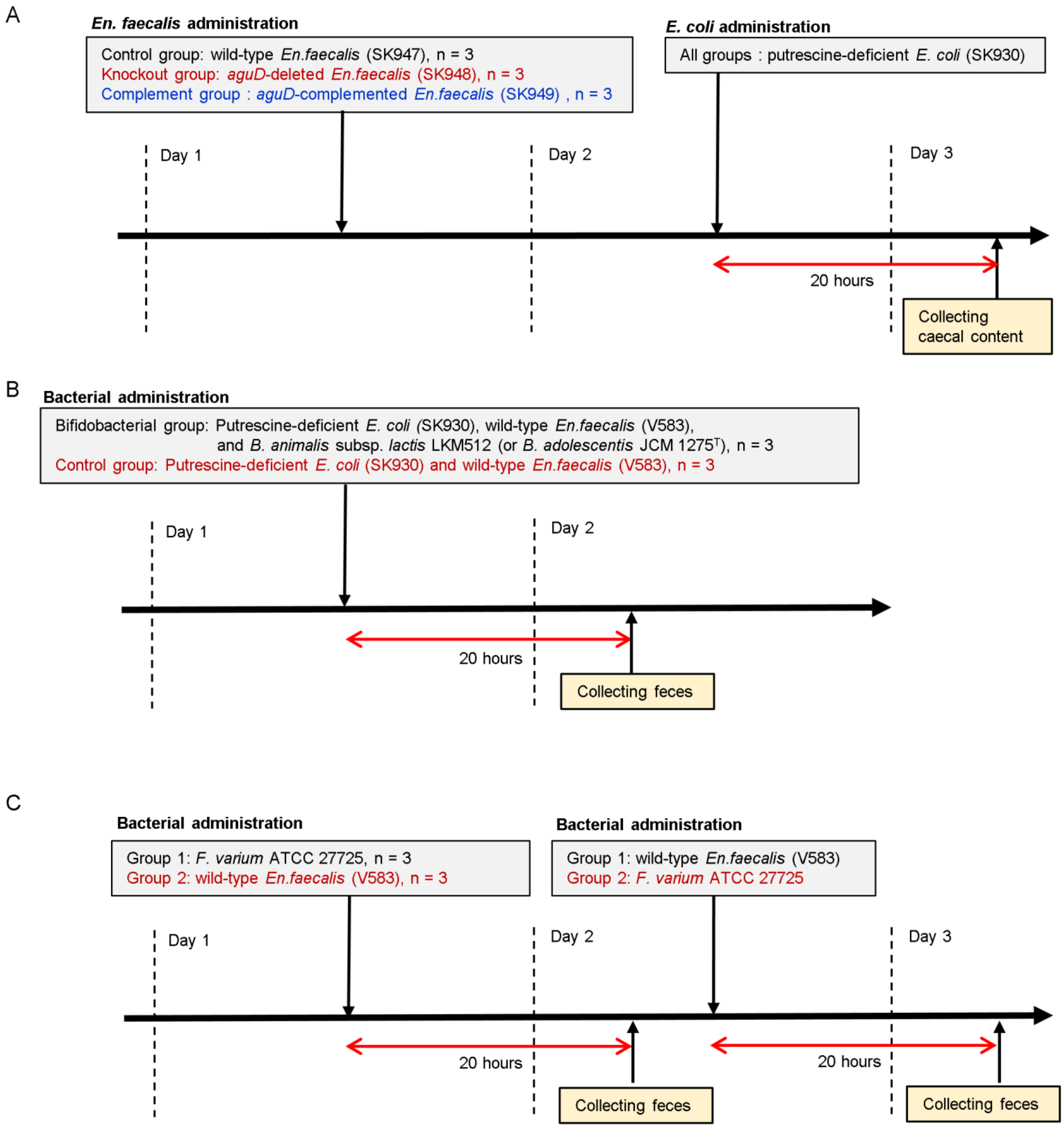

Fig. S11. Outline of gnotobiotic mouse experiments
(**A**) Gnotobiotic mice inoculated with putrescine-deficient *E. coli* (SK930) and *En. faecalis* gene knockout mutants or complementary transformants of the agmatine-putrescine antiporter (aguD). (**B**) Gnotobiotic mice inoculated with putrescine-deficient *E. coli* (SK930), wild-type *En. faecalis* (V583), and *B. animalis* subsp. *lactis* LKM512 (or *B. adolescentis*). (**C**) Gnotobiotic mice inoculated with *F. varium* ATCC27725 and wild-type *En. faecalis* (V583).

Table S1. List of species expressing homologs of the enzymes AdiA and AdiC, as determined by in silico analyses of 126 bacterial strains present in the human gut

	AdiC		AdiA		SpeA		AguD		AguA	
	Bit	Acsestion ID	Bit	Acsestion ID	Bit	Acsestion ID	Bit	Acsestion ID	Bit	Acsestion ID
Actinomyces odontolyticus ATCC 17982										
Bifidobacterium adolescentis ATCC 15703							111	gb|EDN82401.1|		
Bifidobacterium adolescentis L2-32										
Bifidobacterium angulatum DSM 20098										
Bifidobacterium animalis subsp. *lactis* AD011										
Bifidobacterium animalis subsp. *lactis* HN019										
Bifidobacterium breve DSM 20213										
Bifidobacterium catenulatum DSM 16992									189	ref|WP_033513568.1|
Bifidobacterium dentium							107	ref|WP_003839068.1|		
Bifidobacterium gallicum DSM 20093	105	gb|KFI59536.1|								
Bifidobacterium longum DJO10A										
Bifidobacterium longum NCC2705										
Bifidobacterium pseudocatenulatum DSM 20438										
Collinsella aerofaciens ATCC 25986										
Collinsella intestinalis DSM 13280										
Collinsella stercoris DSM 13279										
Alistipes putredinis DSM 17216							103	ref|WP_004329135.1|		
Bacteroides caccae ATCC 43185					545	ref|WP_005679213.1|	108	gb|EDM21916.1|	168	gb|EDM22213.1|
Bacteroides capillosus ATCC 29799					545	ref|WP_007756434.1|	102	ref|WP_007209680.1|	132	ref|WP_040311096.1|
Bacteroides cellulosilyticus DSM 14838					531	ref|WP_007210943.1|			176	gb|EEF87524.1|
Bacteroides coprocola DSM 17136					529	gb|EDV02459.1|			155	gb|EDU99579.1|
Bacteroides coprophilus DSM 18228					531	gb|EEF76458.1|			132	gb|EEF74901.1|
Bacteroides dorei DSM 17855					538	gb|EEB25237.1|			129	gb|EEB24739.1|
Bacteroides eggerthii DSM 20697					541	ref|WP_004291990.1|	103	gb|EEC53335.1|	168	gb|EEC53127.1|
Bacteroides finegoldii DSM 17565					545	ref|WP_007756434.1|	107	gb|EEX43976.1|	162	gb|EEX45314.1|
Bacteroides fragilis 3 1 12										
Bacteroides fragilis NCTC 9343					537	emb|CAH05973.1|	106	emb|CAH06244.1|	161	emb|CAH08166.1|
Bacteroides fragilis YCH46					537	ref|YP_097522.1|	107	ref|YP_097822.1|	159	ref|YP_099665.1|
Bacteroides intestinalis DSM 17393					533	gb|EDV06321.1|			174	gb|EDV02903.1|
Bacteroides ovatus ATCC 8483					547	gb|EDO12412.1|	104	gb|EDO14173.1|	168	gb|EDO12088.1|
Bacteroides plebeius DSM 17135					530	gb|EDY97001.1|			154	gb|EDY96287.1|
Bacteroides sp. 1 1 6										
Bacteroides sp. D1										
Bacteroides sp. D2										
Bacteroides stercoris ATCC 43183					541	gb|EDS14810.1|	105	gb|EDS16535.1|	149	gb|EDS15997.1|
Bacteroides thetaiotaomicron 3731										
Bacteroides thetaiotaomicron 7330										
Bacteroides thetaiotaomicron VPI-5482					550	ref|NP_812306.1|	108	ref|NP_811486.1|	147	ref|NP_809789.1|
Bacteroides uniformis ATCC 8492					527	gb|EDO55269.1|			159	gb|EDO54549.1|
Bacteroides vulgatus ATCC 8482					538	gb|ABR39026.1|	100	gb|ABR41504.1|	130	gb|ABR40853.1|
Bacteroides WH2										
Bacteroides xylanisolvens XB1A					548	emb|CBK67757.1|	106	emb|CBK66780.1|	166	emb|CBK65746.1|
Parabacteroides distasonis ATCC 8503							108	ref|WP_005857294.1|	155	ref|WP_011966340.1|
Parabacteroides johnsonii DSM 18315					538	gb|EEC95655.1|	111	gb|EEC97972.1|	175	gb|EEC96589.1|
Parabacteroides merdae ATCC 43184					541	gb|EDN84738.1|	112	gb|EDN85020.1|	172	gb|EDN86805.1|
Prevotella copri DSM 18205					519	gb|EFB33967.1|			166	gb|EFB35732.1|
Methanobrevibacter smithii ATCC 35061										
Methanobrevibacter smithii DSM 2374										
Methanobrevibacter smithii DSM 2375										
Methanosphaera stadtmanae DSM 3091										
Anaerococcus hydrogenalis DSM 7454										
Anaerofustis stercorihominis DSM 17244										
Anaerostipes caccae DSM 14662										
Anaerotruncus colihominis DSM 17241										
Blautia hansenii DSM 20583										
Blautia hydrogenotrophica DSM 10507										
Bryantella formatexigens DSM 14469										
Butyrivibrio crossotus DSM 2876									301	gb|EFF68065.1|
Catenibacterium mitsuokai DSM 15897									336	gb|EEF93927.1|
Clostridium asparagiforme DSM 15981	105	gb|EEG57719.1|								
Clostridium bartlettii DSM 16795										
Clostridium bolteae ATCC BAA-613										
Clostridium hiranonis DSM 13275										
Clostridium hylemonae DSM 15053										
Clostridium leptum DSM 753										
Clostridium methylpentosum DSM 5476										
Clostridium nexile DSM 1787										
Clostridium ramosum DSM 1402										
Clostridium scindens ATCC 35704	104	gb|EDS08131.1|								
Clostridium sp. L2-50									534	gb|EDO56807.1|
Clostridium sp. M62/1										
Clostridium sp. SS2/1										
Clostridium spiroforme DSM 1552										
Clostridium sporogenes ATCC 15579	204	gb|EDU38352.1|	132	gb|EDU39429.1|			152	gb|EDU37786.1|		
Clostridium symbiosum										
Coprococcus comes ATCC 27758										
Coprococcus eutactus ATCC 27759										
Dorea formicigenerans ATCC 27755	104	ref|WP_005330285.1|								
Dorea longicatena DSM 13814										
Eubacterium biforme DSM 3989										
Eubacterium dolichum DSM 3991										
Eubacterium eligens ATCC 27750									334	gb|ACR72415.1|
Eubacterium hallii DSM 3353										
Eubacterium rectale ATCC 33656									316	gb|ACR77067.1|
Eubacterium rectale DSM 17629									320	emb|CBK89696.1|
Eubacterium ventriosum ATCC 27560									315	emb|CRL40832.1|
Faecalibacterium prausnitzii A2-165										
Faecalibacterium prausnitzii M21/2										
Holdemania filiformis DSM 12042										
Lactobacillus casei ATCC 334	111	dbj|BAN74254.1|	279	ref|WP_011674560.1|						
Lactobacillus delbrueckii subsp. *bulgaricus* ATCC 11842			309	emb|CAI98549.1|						
Lactobacillus reuteri DSM 20016	119	gb|ABQ83632.1|								
Lactococcus lactis subsp. *cremoris* MG1363	102	emb|CAL98875.1|								
Lactococcus lactis subsp. *cremoris*	102	emb|CAB93581.2|								
Lactococcus lactis subsp. *lactis* Il1403	101	ref|NP_268192.1|								
M23A										
Mitsuokella multacida DSM 20544			101	gb|EEX68878.1|						
Parvimonas micra ATCC 33270										
Roseburia intestinalis L1-82									305	gb|EEV01176.1|
Ruminococcus bromii L263										
Ruminococcus gnavus ATCC 29149										
Ruminococcus lactaris ATCC 29176										
Ruminococcus obeum ATCC 29174										
Ruminococcus torques ATCC 27756										
Streptococcus infantarius subsp. *infantarius* ATCC BAA-102										
Streptococcus thermophilus CNRZ1066										
Streptococcus thermophilus LMD-9										
Streptococcus thermophilus LMG 18311										
Subdoligranulum variabile DSM 15176										
Fusobacterium sp. 4 1 13			122	gb|EEO39391.1|						
Fusobacterium varium ATCC 27725	125	gb|EES65190.1|	1009	gb|EES65191.1|						
Citrobacter youngae ATCC 29220	848	gb|EFE05427.1|	1514	gb|EFE05425.1|	1280	gb|EFE06567.1|				
Desulfovibrio piger ATCC 29098					432	gb|EEB32660.1|				
Desulfovibrio piger GOR1					462	WP_040369942.1				
Enterobacter cancerogenus	258	ref|WP_006177175.1|	454	ref|WP_034829451.1|	1292	ref|WP_042321927.1|				
Escherichia coli str. K-12 substr. MG1655	881	ref|NP_418539.1|	1589	ref|NP_418541.2|	1368	ref|NP_417413.1|				
Escherichia fergusonii ATCC 35469	295	emb|CAQ89410.1|	481	emb|CAQ89409.1|	1361	emb|CAQ90371.1|				
Helicobacter pylori HPAG1					325	gb|ABF85039.1|				
Proteus penneri ATCC 35198	113	gb|EEG86360.1|	587	gb|EEG86361.1|	924	gb|EEG85449.1|				
Providencia alcalifaciens DSM 30120	122	gb|EEB44926.1|	1155	gb|EEB45162.1|	1044	gb|EEB44175.1|	101	gb|EEB44240.1|		
Providencia rettgeri DSM 1131	121	gb|EFE55447.1|	1156	gb|EFE52717.1|	1039	gb|EFE53196.1|				
Providencia rustigianii DSM 4541	121	gb|EFB71127.1|	1159	gb|EFB72852.1|	1043	gb|EFB71688.1|				
Providencia stuartii ATCC 25827	117	gb|EDU59312.1|	1170	gb|EDU61801.1|	1044	gb|EDU60243.1|				
Shigella sp. D9										
Akkermansia muciniphila ATCC BAA-835					479	gb|ACD05477.1|	115	gb|ACD03884.1|	204	gb|ACD03962.1|
Victivallis vadensis ATCC BAA-548										

Data from 126 bacterial strains was obtained from article of Yatsunenko et al. (Nature 486: 222-227, 2012)

<100
🟨 100-300
🟧 300-500
🟥 500-

Table S2. Detection of RNA sequences of AdiA, AdiC, AguA, and AguD by metatranscriptomic analysis of human feces

	No. of RNA sequences															
Individual ID	X310763260		X311245214		X316192082		X316701492		X317690558		X317802115		X317822438		X319146421	
	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA	DNA	RNA
AdiA																
K01583*																
Blautia_hansenii|644140017|K01583:K01582	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
Clostridium_leptum|641399920|K01583	6	0	1	0	1	0	1	0	0	0	0	0	9	0	5	0
Clostridium_methylpentosum|644207192|K01583:K01582	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coprococcus_catus|650535763|K01583	3	0	37	0	26	1	29	12	23	0	17	1	3	0	1	0
Eubacterium_rectale|650550223|K01583:K01582	178	29	374	7	629	28	515	6	2	2	33	29	231	69	251	1
Eubacterium_siraeum|642022803|K01583	2	0	117	3	159	0	7	0	10	2	93	2	7	0	0	0
Faecalibacterium_prausnitzii|650556132|K01583	370	0	54	0	97	0	35	0	224	7	172	1	248	0	130	0
Faecalibacterium_prausnitzii|641408861|K01583	122	0	329	2	122	4	70	0	184	3	85	1	266	0	123	0
Faecalibacterium_prausnitzii|646121210|K01583	338	1	98	0	235	2	37	3	236	8	96	3	688	4	219	0
Faecalibacterium_prausnitzii|646120999|K01583	226	4	30	2	119	2	2	0	38	17	25	0	371	61	156	2
Pseudoflavonifractor_capillosus|641048424|K01583	0	0	4	0	1	0	1	0	0	0	1	0	0	0	2	1
Roseburia_intestinalis|650568492|K01583	30	0	43	3	10	7	4	0	19	0	31	15	6	0	0	0
Ruminococcus_albus|647433160|K01583	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ruminococcus_lactaris|642852867|K01583:K01582	49	0	0	0	2	0	106	0	0	0	26	0	0	0	83	0
Ruminococcus_obeum|650580233|K01583	30	0	144	2	10	0	65	0	42	0	4	0	6	0	9	0
Ruminococcus_obeum|641008143|K01583	107	4	20	0	93	0	40	0	33	1	7	0	2	0	101	0
Ruminococcus_torques|650582987|K01583	24	1	43	0	91	0	46	0	51	0	7	0	15	0	41	0
Streptococcus_australis|650325720|K01583:K01582	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus_sanguinis|650294352|K01583:K01582	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Subdoligranulum_variabile|644151278|K01583	19	0	20	0	50	0	12	0	20	1	28	0	11	1	26	0
K01584*																
Escherichia_coli|641619399|K01584	14	173	39	289	16	195	7	315	3	221	108	172	3	451	2	493
Escherichia_coli|644756906|K01584	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxalobacter_formigenes|646260576|K01584:K01618	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
K01585*																
Akkermansia_muciniphila|642613717|K01585	0	0	29	0	0	0	34	0	1	0	3	5	129	5	5	0
Alistipes_putredinis|642181069|K01585	403	5	277	1	94	0	8	0	27	14	142	5	494	55	148	6
Alistipes_shahii|650520745|K01585	276	14	348	10	166	4	306	19	37	7	408	179	176	22	827	94
Anaerofustis_stercorihominis|642325472|K01585:K01582	0	2	0	0	0	1	0	2	0	0	0	0	4	9	0	7
Bacteroides_caccae|641003695|K01585	10	5	54	14	6	0	106	42	7	15	15	10	45	54	1	0
Bacteroides_cellulosilyticus|644166404|K01585	502	541	19	46	31	27	69	22	7	4	117	43	89	176	335	488
Bacteroides_dorei|646230453|K01585	50	1	106	3	170	1	63	2	93	84	151	22	47	6	0	0
Bacteroides_eggerthii|650041221|K01585	25	33	382	284	30	13	5	0	90	82	1	0	4	0	0	0
Bacteroides_finegoldii|643132120|K01585	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0
Bacteroides_fragilis|637225963|K01585	39	10	65	10	16	2	0	0	1	1	0	0	0	2	137	17
Bacteroides_fragilis|645276602|K01585	1	1	0	0	0	0	0	1	3	0	1	2	0	0	0	0
Bacteroides_intestinalis|642809518|K01585	3	1	1	1	0	0	7	8	12	6	4	0	178	504	1	0
Bacteroides_ovatus|641382330|K01585	11	4	5	1	32	4	15	0	9	10	34	18	1	12	119	26
Bacteroides_salanitronis|649968362|K01585	4	0	0	4	0	0	0	0	1	9	0	0	0	0	0	0
Bacteroides_stercoris|642198809|K01585	0	0	2	0	1	3	801	84	28	29	0	0	0	0	0	0
Bacteroides_thetaiotaomicron|637411863|K01585	64	21	38	24	21	13	66	21	3	24	23	18	69	351	52	188
Bacteroides_uniformis|641386981|K01585	593	402	468	269	210	282	494	100	138	129	115	71	283	1775	352	1377
Bacteroides_vulgatus|640761455|K01585	502	33	199	19	101	0	569	6	489	544	131	26	813	57	1	0
Bacteroides_xylanisolvens|650525678|K01585	33	6	55	7	33	3	2	0	11	10	57	12	1	14	34	5
Bilophila_wadsworthia|650058069|K01585	51	1	6	19	2	1	19	6	3	0	14	6	24	31	52	29
Clostridium_bartlettii|642163716|K01585:K01582	0	0	0	0	0	0	0	0	0	2	2	0	2	1	0	0
Desulfovibrio_desulfuricans|643581697|K01585	0	0	8	0	0	0	0	0	0	0	0	0	0	0	1	1
Desulfovibrio_piger|643139729|K01585	2	0	7	2	0	0	46	1	0	0	0	0	0	0	0	0
Escherichia_coli|637357372|K01585	13	218	34	194	7	224	2	259	1	159	109	136	5	331	0	401
Odoribacter_splanchnicus|649961909|K01585	44	6	23	16	8	1	23	0	24	11	14	5	51	2	43	2
Parabacteroides_johnsonii|643157369|K01585	68	49	11	21	9	2	74	177	0	3	3	9	5	3	0	0
Parabacteroides_merdae|641046169|K01585	1	0	90	268	92	35	0	2	24	23	1	5	309	244	42	99
Prevotella_copri|644148832|K01585	0	0	1	0	286	101	0	0	2	0	0	0	0	0	0	0
K02626*																
Methanobrevibacter_smithii|644142724|K02626	0	0	47	38	20	9	7	6	13	6	0	0	0	0	27	26
Methanosphaera_stadtmanae|637847217|K02626	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	3
Parabacteroides_distasonis|640765494|K02626	37	26	21	126	14	15	15	7	4	3	0	15	46	31	23	23
AdiC																
K03759*																
Escherichia_coli|648567877|K03759	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
K03756*																
Escherichia_coli|648381214|K03756	1	20	6	39	2	25	0	44	1	21	13	13	0	34	0	47
Escherichia_coli|648535536|K03756	6	81	15	104	2	62	1	175	0	104	50	57	3	160	0	164
Dialister_invisus|646034334|K03756	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dialister_invisus|646033313|K03756	0	0	16	0	0	0	0	0	0	0	0	0	15	0	128	0
Haemophilus_parainfluenzae|650599334|K03756	0	0	2	0	0	0	0	0	27	71	0	1	0	2	0	0
Haemophilus_parainfluenzae|651496018|K03756	0	0	0	0	0	0	0	0	3	13	0	0	0	0	3	0
Sutterella_wadsworthensis|650088830|K03756	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sutterella_wadsworthensis|650088831|K03756	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Veillonella_atypica|648784587|K03756	0	0	0	0	6	5	2	0	0	0	2	5	0	0	0	0
Veillonella_atypica|648785563|K03756	0	0	0	0	4	0	0	0	4	0	4	0	0	0	0	0
Veillonella_dispar|644360141|K03756	0	0	0	0	0	0	0	0	33	0	3	0	0	0	0	0
Veillonella_parvula|646432871|K03756	0	0	0	0	0	0	0	0	22	0	9	1	0	0	1	0
AguA																
K01235*	420	9	19	11	28	9	55	0	1	0	107	32	107	19	306	130
Bacteroides_cellulosilyticus|644168268|K01235	420	9	19	11	28	9	55	0	1	0	107	32	107	19	306	130
Bacteroides_dorei|643119634|K01235	11	0	71	6	134	17	48	17	73	14	133	56	25	0	0	0
Bacteroides_eggerthii|643096546|K01235	26	2	305	63	31	2	0	0	88	89	0	0	0	0	0	0
Bacteroides_eggerthii|650042560|K01235	2	0	349	28	20	4	0	0	74	23	0	0	0	0	0	0
Bacteroides_intestinalis|642812315|K01235	63	1	4	2	4	2	22	0	14	5	15	6	135	24	45	14
Bacteroides_ovatus|651358078|K01235	0	0	0	0	24	0	6	0	1	3	56	22	0	0	174	16
Bacteroides_ovatus|641380680|K01235	0	0	0	0	4	0	6	0	0	0	0	0	146	49	0	0
Bacteroides_ovatus|647455895|K01235	2	0	7	1	6	0	0	0	0	0	0	2	0	0	0	0
Bacteroides_thetaiotaomicron|637411075|K01235	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Bacteroides_vulgatus|647438526|K01235	425	18	147	23	56	2	85	3	377	48	105	28	746	199	2	0
Bacteroides_xylanisolvens|650527454|K01235	32	8	53	7	54	0	2	0	5	0	47	20	0	0	0	0
Bacteroides_xylanisolvens|647441259|K01235	0	0	10	2	10	0	0	0	0	0	2	1	0	0	0	0
Clostridium_hathewayi|646040426|K01235	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	11
Enterococcus_faecium|647721935|K01235	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactococcus_lactis|646463857|K01235	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactococcus_lactis|640101603|K01235	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Prevotella_copri|644150454|K01235	0	0	0	0	273	35	0	0	0	0	0	0	0	1	0	0
Ruminococcus_albus|647432835|K01235	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AguD																
K20265*																
Not detected																

* KO number authorized by KEGG database (http://www.genome.jp/kegg/)

Results were modified from Franzosa et al. (27) (www.pnas.org/cgi/doi/10.1073/pnas.1319284111). Columns are labeled as subject ID, DNA as fecal metagenomes, and RNA as fecal metatranscriptomes of 8 control subjects. Each row corresponds to annotated species name, IMG gene ID, and colon-delimited KEGG Orthogroup assignments. Values are count data representing read hits for these genes.

Table S3. List of bacteria used for screening of polyamine producing bacteria

Strains	Phylum
Alistipes putredinis JCM16772	Bacteroidetes
Bacteroides caccae JCM9498	Bacteroidetes
Bacteroides thetaiotaomicron JCM 5827^T	Bacteroidetes
Bacteroides uniformis JCM5828	Bacteroidetes
Bacteroides vulgatus JCM 5826^T	Bacteroidetes
Bifidobacterium adolescentis JCM 1275^T	Actinobacteria
Bifidobacterium longum JCM 1217^T	Actinobacteria
Blautia hansenii JCM14655^T	Firmicutes
Clostridium leptum DSM753	Firmicutes
Enterococcus faecalis JCM 5803^T	Firmicutes
Escherichia coli JCM 5491	Proteobacteria
Parabacteroides johnsonii JCM13406	Bacteroidetes
Parabacteroides merdae JCM9497	Bacteroidetes
Streptococcus thermophiles NBRC13957	Firmicutes

Table S4. Strains, plasmids, and oligonucleotides used in this study

Strain, plasmid, or oligonucleotide	Characteristic or sequence	Source or reference
Strains		
Escherichia coli K-12		
JW4076	ME9062 except Δ*adiC* :: FRT-*kan*$^+$-FRT	Baba et al. 2006
JW5731	ME9062 except Δ*adiA* :: FRT-*kan*$^+$-FRT	Baba et al. 2006
LKM10096	pBeloBAC11/MG1655	This study
LKM10097	pBelobac11/SK903	This study
LKM10100	pBelobac11-*adiC*$^+$/SK903	This study
ME9062	*rrnB* Δ*lacZ4787* *hsdR514* Δ(*araBAD*)*567* Δ(*rhaBAD*)*568* *rph-1*	Baba et al. 2006
MG1655	F$^-$ prototrophic	C. A. Gross
SK900	MG1655 except Δ*adiA* :: FRT-*kan*$^+$-FRT	This study
SK901	MG1655 except Δ*adiA* ::FRT	This study
SK902	MG1655 except Δ*adiC* :: FRT-*kan*$^+$-FRT	This study
SK903	MG1655 except Δ*adiC* ::FRT	This study
SK912	pBelobac11-*adiA*$^+$/SK901	This study
SK914	pBelobac11/SK901	This study
SK930	pBelobac11/MG1655 except Δ*speB* ::FRT Δ*speC* ::FRT Δ*speF* ::FRT	This study
Enterococcus faecalis		
SK932	pLT06-Δ*aguD* /V583	This study
SK934	V583 except Δ*aguD* *lacZ*$^+$ *cat*$^+$ *pheS*$^+$ *aguD*$^+$	This study
SK937	V583 except Δ*aguD*	This study
SK947	pLZ12/V583	This study
SK948	pLZ12/SK937	This study
SK949	pLZ12-*aguB*$^+$*D*$^+$/SK937	This study
V583	Clinical isolate, TIGR sequence strain; Van^R	American Type Culture Collection
Plasmids		
harbouring genes of *E. coli* K-12 MG1655		
pBelobac11	Mini-F replicon *cat*$^+$	New England Biolabs Inc., Beverly, Massachusetts, USA
pBelobac11-*adiA*$^+$	Mini-F replicon *cat*$^+$ *adiA*$^+$	This study
pBelobac11-*adiC*$^+$	Mini-F replicon *cat*$^+$ *adiC*$^+$	This study
harbouring genes of *En. faecalis* V583		
pLT06	*repA* -ts replicon *pheS*$^+$-*cat*$^+$-*lacZ*$^+$	Thurlow et al. 2009
pLT06-Δ*aguD*	*repA* -ts replicon Δ*aguD* *pheS*$^+$ *cat*$^+$ *lacZ*$^+$	This study
pLZ12	pSH71 replicon *cat*$^+$	Perez-Casal et al. 1991
pLZ12-*aguB*$^+$*D*$^+$	pSH71 replicon *cat*$^+$ *aguB*$^+$*D*$^+$	This study
Oligonucleotides		
adiA_300bp-up_for	ccgtgccggcacgttTCAAGTAGTCGTTTTTACAG	
adiA_term_rev	ggatgcagcccggttTTACGCTTTCACGCACATAA	
adiC_300bp-up_for	ccgtgccggcacgttAAAAGTTGAAAAGCGAAAAC	
adiC_term_rev	ggatgcagcccggttTTAATCTTTGCTTATTGGTG	
Clone_agcB+0.5K_FWD	tgaattcattaggatcGTGCGGTCATAAAGGTCTGA	
Clone_agcD+COMPL	aggaagatctggatcTTAGCCATTTTCTTTCGTTCCCTT	