

SUPPLEMENTARY TABLES AND FIGURES

Table S1: Isolated microorganisms from three fecal samples from controls on five different media.

	YCFAG			SCH	
	$\Delta 0$ +AAA +FS	ΔV +AAA +FS	ΔS +AAA +FS	SCH	SCH +FS
ACTINOBACTERIA					
<i>Bifidobacteriaceae</i>					
<i>Bifidobacterium catenulatum/</i> <i>pseudocatenulatum</i>	N	N	N	N	N
<i>Bifidobacterium longum</i>	N	N	N	N	
<i>Coriobacteriaceae</i>					
<i>Collinsella aerofaciens</i>	N				
<i>Corynebacteriaceae</i>					
<i>Corynebacterium sp.</i>	O	O			O
<i>Corynebacterium aurimucosum</i>		O	O		
<i>Corynebacterium striatum</i>		O	O		
BACTEROIDETES					
<i>Bacteroidaceae</i>					
<i>Bacteroides caccae</i>		N			
<i>Bacteroides cellulosilyticus</i>		N	N		
<i>Bacteroides fragilis</i>	N				
<i>Bacteroides ovatus</i>	N			N	
<i>Bacteroides thetaiotaomicron</i>			N		
<i>Bacteroides uniformis</i>		N		N	
<i>Bacteroides vulgatus</i>	N	N	N	N	
<i>Tannerellaceae</i>					
<i>Parabacteroides distasonis</i>	N				
FIRMICUTES					
<i>Bacillaceae</i>					
<i>Bacillus sp.</i>			O		
<i>Bacillus pumilus</i>	O	O			
<i>Enterococcaceae</i>					
<i>Enterococcus faecium</i>	O/N	O/N	O/N	O	O/N
<i>Enterococcus mundtii</i>	O		O		O
<i>Erysipelotrichaceae</i>					
<i>Clostridium innocuum</i>		N		N	
<i>Staphylococcaceae</i>					
<i>Staphylococcus sp.</i>					O
<i>Staphylococcus parasanguinis</i>	O				
PROTEOBACTERIA					
<i>Enterobacteriaceae</i>					
<i>Citrobacter amalonaticus</i>				O	
<i>Escherichia coli</i>	O/N	O/N	O/N	O/N	O/N
EUKARYOTES					
<i>Candida albicans</i>				O	
TOTAL N° OF DIFFERENT ISOLATES (O/N)	6/9	6/9	6/7	4/7	5/3

YCFAG: Yeast Casitone Fatty Acid Glucose medium; $\Delta 0$: medium unchanged; ΔV : medium without vitamins; ΔS : medium without short chain fatty acids; FS: fecal suspension; AAA: aromatic amino acids; SCH: Schaedler medium; O: isolated from fecal samples on corresponding medium after aerobic incubation (37 °C for 2 to 5

days); N: isolated from fecal samples on corresponding medium after anaerobic incubation (37 °C for 5 to 7 days).

Table S2: Correlation between the abundance of the bacterial taxa, as assessed by means of qPCR, and estimated glomerular filtration rate (eGFR).

	eGFR	
	<i>r_s</i>	<i>p</i> -value
Total number of bacterial 16S rRNA gene copies	0.178	0.053
<i>Akkermansia muciniphila</i>	0.131	0.147
<i>Bacteroides</i> spp.	0.118	0.193
<i>Bifidobacterium</i> spp.	0.255	0.004
<i>Butyricoccus</i> spp.	0.328	<0.001
<i>Clostridioides difficile</i>	-0.137	0.129
<i>Enterobacteriaceae</i>	-0.201	0.027
<i>Escherichia coli</i>	-0.252	0.005
<i>Faecalibacterium prausnitzii</i>	0.220	0.014
<i>Lactobacillus</i> spp.	0.137	0.131
<i>Roseburia</i> spp.	0.324	<0.001
<i>Streptococcus</i> spp.	0.143	0.127

r_s: Spearman's rank correlation coefficient

Table S3: Media composition (for 1 L)

Products	YCFAG +AAA+FS	YCFAG ΔG +AAA +FS	YCFAG ΔC +AAA +FS	YCFAG ΔM +AAA +FS	YCFAG ΔV +AAA +FS	YCFAG ΔS +AAA +FS	SCH +AAA +FS	1203a
Anaerobe basal broth (Thermo Fisher Scientific) (g)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	35.4
Bacto™ agar (BD)° (g)	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Bacto™ Casitone (BD) (g)	10.0	10.0	N/A	10.0	10.0	10.0	N/A	N/A
Fecal suspension, sterile* (ml)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	N/A
Glucose (g)	4.50	N/A	4.50	4.50	4.50	4.50	N/A	N/A
L-Cystein-HCl* (g)	1.0	1.0	1.0	1.0	1.0	1.0	N/A	N/A
L-Phenylalanine* (mg)	413.0	413.0	413.0	413.0	413.0	413.0	413.0	N/A
L-Tryptophan* (mg)	511.0	511.0	511.0	511.0	511.0	511.0	511.0	N/A
L-Tyrosine* (mg)	453.0	453.0	453.0	453.0	453.0	453.0	453.0	N/A
NaHCO ³ * (g)	4.0	4.0	4.0	4.0	4.0	4.0	N/A	N/A
Resazurin (of 1 mg/ml) (ml)	1.0	1.0	1.0	1.0	1.0	1.0	N/A	N/A
SCH anaerobe broth (g)	N/A	N/A	N/A	N/A	N/A	N/A	26.4	N/A
Yeast extract (g)	2.5	2.5	2.5	2.5	2.5	2.5	N/A	N/A
Minerals								
CaCl ₂ (mg)	90.0	90.0	90.0	N/A	90.0	90.0	N/A	N/A
K ₂ HPO ₄ (mg)	450.0	450.0	450.0	N/A	450.0	450.0	N/A	N/A
KH ₂ PO ₄ (mg)	450.0	450.0	450.0	N/A	450.0	450.0	N/A	N/A
MgSO ₄ ·7H ₂ O (mg)	90.0	90.0	90.0	N/A	90.0	90.0	N/A	N/A
NaCl (mg)	900.0	900.0	900.0	N/A	900.0	900.0	N/A	N/A
Vitamins – Growth factors								
Biotin* (μg)	10.0	10.0	10.0	10.0	N/A	10.0	N/A	N/A
Cobalamin* (μg)	10.0	10.0	10.0	10.0	N/A	10.0	N/A	N/A
Haemin (mg)	10.0	10.0	10.0	10.0	N/A	10.0	N/A	N/A
Folic acid* (μg)	50.0	50.0	50.0	50.0	N/A	50.0	N/A	N/A
<i>p</i> -aminobenzoic acid* (μg)	30.0	30.0	30.0	30.0	N/A	30.0	N/A	N/A
Pyridoxine-HCl* (μg)	150.0	150.0	150.0	150.0	N/A	150.0	N/A	N/A
Riboflavin* (μg)	50.0	50.0	50.0	50.0	N/A	50.0	N/A	N/A
Thiamin* (μg)	50.0	50.0	50.0	50.0	N/A	50.0	N/A	N/A
Short chain fatty acids								
Acetate (ml)	1.948	1.948	1.948	1.948	1.948	N/A	N/A	N/A

Propionate (ml)	0.658	0.658	0.658	0.658	0.658	N/A	N/A	N/A
Isobutyrate (ml)	0.088	0.088	0.088	0.088	0.088	N/A	N/A	N/A
Isovalerate (ml)	1.021	1.021	1.021	1.021	1.021	N/A	N/A	N/A
Valerate (ml)	1.021	1.021	1.021	1.021	1.021	N/A	N/A	N/A
pH	6.8 ±0.1	6.8 ±0.1	6.8 ±0.1	6.8 ±0.1	6.8 ±0.1	6.8 ±0.1	6.8 ±0.1	6.8 ±0.1

YCFAG: Yeast Casitone Fatty Acid Glucose; SCH: Schaedler medium; AAA: aromatic amino acids (L-phenylalanine, L-tyrosine, and L-tryptophan); FS: fecal suspension; ΔG: medium without glucose; ΔC: medium without casitone; ΔM: medium without minerals; ΔV: medium without vitamins; ΔS: medium without short chain fatty acids; BD: Becton Dickinson; N/A: not applicable; *: added after autoclaving; °: added to prepare agar plates; all products were purchased from Sigma Aldrich unless stated otherwise.

Table S4: Primers, probes and thermal cycling conditions for all performed qPCRs

qPCR (Target gene)	Pos/ Neg (%) ^o	Sequence	length (bp)	Primer/ Probe (μ M)	MgCl ₂ (mM)	Thermal cycling conditions				LOQ LOD (log ₁₀)	Ref.
						Pre- step	Amplification	Melting	Cooling		
Total bacterial 16S rRNA gene copies (16S rRNA gene)	96/0	F: ACTCCTACGGGAGGCAGCACT R: GTAATTCGCGGCTGCTGGCAC	194-200	0.5 0.5	3	10' 95°C	45x 15'' 95°C 40'' 60°C 30'' 72°C	5'' 95°C 1' 55°C 97°C*	30'' 40°C	5.04 4.80	[1]
<i>Akkermansia muciniphila</i> (16S rRNA gene)	89/11	F: CAGCACGTGAAGGTGGGGAC R: CCTTGCGGTGGCTTCAGAT	329	0.5 0.5	2	10' 95°C	40x 15'' 95°C 40'' 64°C 30'' 72°C	5'' 95°C 1' 60°C 97°C*	30'' 40°C	4.11 3.53	[1, 2]
<i>Bacteroides</i> spp. (16S rRNA gene)	100/0	F: GGGTTTAAAGGAGCGTAGG R: CTACACCACGAATTCCGCCT P: FAM ^a -TAAGTCAGTTGTGAAAGTTGCGGCTC-TAMRA ^b	116	0.3 0.3 0.2	0	10' 95°C	45x 30'' 95°C 45'' 60°C	N/A	30'' 40°C	3.32 2.75	[3, 4]
<i>Bifidobacterium</i> spp. (16S rRNA gene)	100/0	F: GAATAGCTCCTGAAAACG R: ATAGGACGCGACCCCA P: FAM ^a -TGGTAATGCCGGATGCTCC-TAMRA ^b	99	0.3 0.3 0.2	0	10' 95°C	45x 15'' 95°C 60'' 62°C	N/A	30'' 40°C	2.69 1.75	Based on [4]
<i>Butyrivibrio</i> spp. (16S rRNA gene)	100/0	F: ACCTGAAGAATAAGCTCC R: GATAACGCTTGCTCCCTACGT	69	0.5 0.5	3	10' 95°C	45x 15'' 95°C 40'' 58°C 30'' 72°C	5'' 95°C 1' 55°C 97°C*	30'' 40°C	2.59 2.51	[5]
<i>Clostridioides difficile</i> (16S rRNA gene)	9/91	F: GCAAGTTGAGCGATTACTTCGGT R: GTACTGGCTCACCTTTGATATTYAAGAG P: FAM ^a -TGCCTCTCAAATATATTATCCCGTATTAG-TAMRA ^b	155	0.2 0.2 0.2	0	10' 95°C	45x 5'' 95°C 50'' 56°C	N/A	30'' 40°C	3.26 2.27	[6]
<i>Enterobacteriaceae</i> spp. (16S rRNA gene)	97/0	F: CATTGACGTTACCCGAGAGAAGC R: CTCTACGAGACTCAAGCTTGC	190	0.5 0.5	3	10' 95°C	45x 15'' 95°C 40'' 63°C 30'' 72°C	5'' 95°C 1' 55°C 97°C*	30'' 40°C	3.50 3.47	[1, 7]
<i>Escherichia coli</i> (uidA gene)	97/3	F: CAACGAACTGAAGTGGCAGA R: CATTACGCTGCGATGGAT P: FAM ^a -TATCCCGCCGGGAATGGTGA-TAMRA ^b	121	0.3 0.3 0.2	0	10' 95°C	45x 15'' 95°C 60'' 64°C	N/A	30'' 40°C	4.67 4.20	[8, 9]
<i>Faecalibacterium prausnitzii</i> (16S rRNA gene)	100/0	F: GGAGGAAGAAGGTCTTCGG R: AATTCGCGCTACCTCTGCACT	248	0.5 0.5	3	10' 95°C	45x 15'' 95°C 40'' 60°C 30'' 72°C	5'' 95°C 1' 55°C 97°C*	30'' 40°C	3.66 2.43	[10, 11]
<i>Lactobacillus</i> spp. (16S rRNA gene)	90/9	F: AGCAGTAGGGAATCTTCCA R: CACCGCTACACATGGAG	340-346	0.3 0.3	2	10' 95°C	45x 15'' 95°C 40'' 58°C 30'' 72°C	5'' 95°C 1' 55°C 97°C*	30'' 40°C	6.05 4.12	[12]
<i>Roseburia</i> spp. (16S rRNA gene)	99/0	F: TACTGCATTGGAACTG R: CGGCACCGAAGAGCAAT	230	0.5 0.5	3	10' 95°C	45x 15'' 95°C 40'' 60°C 30'' 72°C	5'' 95°C 1' 55°C 97°C*	30'' 40°C	2.37 2.22	[13]
<i>Streptococcus</i> spp. (16S rRNA gene)	81/12	F: GAAGAATTGCTTGAATTGGTTGAA R: GGACGGTAGTTGTTGAAGAATGG	559	0.5 0.5	3	10' 95°C	45x 15'' 95°C 40'' 60°C 30'' 72°C	5'' 95°C 1' 55°C 97°C*	30'' 40°C	5.11 4.45	[14]

F: forward primer sequence; R: reverse primer sequence; P: probe; bp: basepair; Ref.: reference; a: 6-carboxyfluorescein, fluorescence reporter dye; b: 5(6)-carboxy-tetramethylrhodamine, fluorescence quencher dye; *: continuous rate at 0.02°C/sec; °: percentage of positive and negative qPCR signals; N/A: not applicable.

Table S5: qPCR specificity

Bacterial species test panel (Origin)	TOT BACT	AKKMUC	BAT spp.	BIF spp.	BUT spp.	CLSDIF	ENT spp.	ESCCOL	FAEPRA	LAB spp.	ROS spp.	STR spp.
ACTINOBACTERIA												
Bifidobacteriaceae												
Bifidobacterium animalis (LBR 011205)	+	-	-	+	-	-	-	-	-	-	-	-
Bifidobacterium bifidum (LMG 11041 ¹)	+	-	-	+	-	-	-	-	-	-	-	-
Bifidobacterium breve (LBR 070975)	+	-	-	+	-	-	-	-	-	-	-	-
Bifidobacterium catenulatum (LBR 0715165)	+	-	-	+	-	-	-	-	-	-	-	-
Bifidobacterium dentium (LBR 710466)	+	-	-	+	-	-	-	-	-	-	-	-
Bifidobacterium longum (LMG 13197T)	+	-	-	+	-	-	-	-	-	-	-	-
<u>Bifidobacterium pseudocatenulatum (LBR 0715206)</u>	+	-	-	+	-	-	-	-	-	-	-	-
BACILLUS												
Enterococcaceae												
Enterococcus durans (LBR 0715214)	+	-	-	-	-	-	-	-	-	-	-	-
<u>Enterococcus faecalis (LBR 0715156)</u>	+	-	-	-	-	-	-	-	-	-	-	-
Enterococcus faecalis (LBR 0715102)	+	-	-	-	-	-	-	-	-	-	-	-
Enterococcus faecium (LBR 0715104)	+	-	-	-	-	-	-	-	-	-	-	-
Lactobacillaceae												
Lactobacillus acidophilus (LBR 011207)	+	-	-	-	-	-	-	-	-	+	-	-
Lactobacillus buchneri (LBR 0715175)	+	-	-	-	-	-	-	-	-	+	-	-
Lactobacillus fermentum (0708283)	+	-	-	-	-	-	-	-	-	+	-	-
Lactobacillus gasseri (LBR 070934)	+	-	-	-	-	-	-	-	-	+	-	-
<u>Lactobacillus jensenii (LMG 6414¹)</u>	+	-	-	-	-	-	-	-	-	+	-	-
Lactobacillus paracasei (LBR 130272)	+	-	-	-	-	-	-	-	-	+	-	-
Lactobacillus rhamnosus (LBR 020586)	+	-	-	-	-	-	-	-	-	+	-	-
BACTEROIDETES												
Bacteroidaceae												

Streptococcaceae

[illegible]

PROTEOBACTERIA

Enterobacteriaceae

<i>Citrobacter freundii</i> (LBR 010201)	+	-	-	-	-	-	+	-	-	-	-	-
<i>Enterobacter cloacae</i> (LBR 0715229)	+	-	-	-	-	-	+	-	-	-	-	-
<i>Escherichia coli</i> (LMG 2092 ^T)	+	-	-	-	-	-	+	+	-	-	-	-
<u><i>Escherichia coli</i> (ATCC 25922)</u>	+	-	-	-	-	-	+	+	-	-	-	-
<i>Escherichia fergusonii</i> (LBR 70436)	+	-	-	-	-	-	+	-	-	-	-	-
<i>Escherichia fergusonii</i> (LBR 111141)	+	-	-	-	-	-	+	-	-	-	-	-
<i>Escherichia hermanii</i> (LBR 130262)	+	-	-	-	-	-	+	-	-	-	-	-
<i>Escherichia hermanii</i> (LBR 111137)	+	-	-	-	-	-	+	-	-	-	-	-
<i>Klebsiella oxytoca</i> (LBR 010202)	+	-	-	-	-	-	+	-	-	-	-	-
<i>Pseudodescherichia vulneris</i> (LBR 111139)	+	-	-	-	-	-	+	-	-	-	-	-
<i>Shigella boydii</i> (LBR 010146)	+	-	-	-	-	-	+	+	-	-	-	-
<i>Shigella boydii</i> (LBR 010152)	+	-	-	-	-	-	+	+	-	-	-	-
<i>Shigella flexneri</i> (LBR 070578)	+	-	-	-	-	-	+	+	-	-	-	-
<i>Shigella flexneri</i> (LBR 220544)	+	-	-	-	-	-	+	+	-	-	-	-
<i>Shigella sonnei</i> (LBR 170413)	+	-	-	-	-	-	+	+	-	-	-	-
Yersiniaceae												
<i>Yersinia enterocolitica</i> (LBR 070622)	+	-	-	-	-	-	+	-	-	-	-	-

VERRUCOMICROBIA

Akkermansiaceae

[illegible]

TOT BACT: Total bacteria; AKKMUC: *Akkermansia muciniphila*; BAT spp.: *Bacteroides* spp.; BIF spp.: *Bifidobacterium* spp.; BUT spp.: *Butyricicoccus* spp.; CLSDIF: *Clostridioides difficile*; ENT spp.: *Enterobacteriaceae* spp.; ENC spp.: *Enterococcus* spp.; ESCCOL: *Escherichia coli*; FAEPR: *Faecalibacterium prausnitzii*; LACT spp.: *Lactobacillus* spp.; ROS spp.: *Roseburia* spp.; STR spp.: *Streptococcus* spp.; +: qPCR amplification; -: no qPCR amplification; gray: expected to be positive; *: cycle of amplification below limit of detection; bacterial strain underlined: strain used to prepare a standard tenfold dilution series.

		YCFAG				YCFAG				YCFAG				YCFAG				YCFAG				SCH								
		$\Delta 0$	$\Delta 0$ +AAA	$\Delta 0$ +FS	$\Delta 0$ +AAA +FS	ΔG	ΔG +AAA	ΔG +FS	ΔG +AAA +FS	ΔC	ΔC +AAA	ΔC +FS	ΔC +AAA +FS	ΔM	ΔM +AAA	ΔM +FS	ΔM +AAA +FS	ΔV	ΔV +AAA	ΔV +FS	ΔV +AAA +FS	ΔS	ΔS +AAA	ΔS +FS	ΔS +AAA +FS	SCH	SCH +AAA	SCH +FS	SCH +AAA +FS	
ANAEROBES	<i>Bacteroides fragilis</i>																													
	<i>B. thetaiotaomicron</i>																													
	<i>Bifidobacterium adolescentis</i>																													
	<i>B. bifidum</i>																													
	<i>B. longum</i>																													
	<i>Clostridioides difficile</i>																													
	<i>Clostridium sporogenes</i>																													
	<i>Lactobacillus acidophilus</i>																													
	<i>L. paracasei</i>																													
<i>Paraclostridium bifermentans</i>																														
AEROBES	<i>Escherichia coli</i>																													
	<i>Staphylococcus epidermidis</i>																													

Figure S1. Ability of the 12 bacterial species of the test panel to grow on 28 different media. Aerobic incubation at 37 °C for 2 days and anaerobic incubation at 37 °C for 7 days; Red: no growth; Green: growth; YCFAG: Yeast Casitone Fatty Acid Glucose medium; $\Delta 0$: medium unchanged; ΔG : medium without glucose; ΔC : medium without casitone; ΔM : medium without minerals; ΔV : medium without vitamins; ΔS : medium without short chain fatty acids; SCH: Schaedler medium; FS: fecal suspension; AAA: aromatic amino acids.

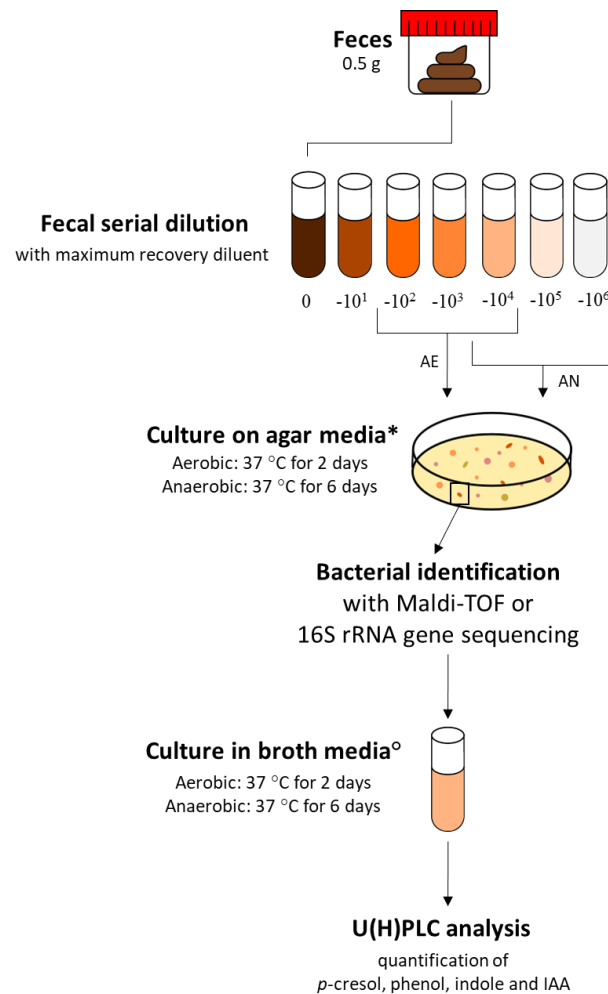


FIGURE S2. Isolation and identification of protein-bound uremic toxin (PBUT) precursor-generating bacteria. AE: aerobic; AN: anaerobic; IAA: indole-3-acetic acid; HPLC: High performance liquid chromatography; UPLC: Ultra performance liquid chromatography, *: three different media used: 1) Yeast Casitone Fatty Acid Glucose medium supplemented with aromatic amino acids and sterile fecal suspension, 2) Schaedler medium, and 3) 1203a medium; °: Yeast Casitone Fatty Acid Glucose broth medium supplemented with aromatic amino acids.

Supplementary References

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