

Molecular epidemiology and comparative genomics of *Campylobacter concisus* strains from saliva, faeces and gut mucosal biopsies in inflammatory bowel disease

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Supplementary material

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Supplementary figure 1: A phylogenetic tree depicting the genetic relatedness of 113 *C. concisus* isolates. The tree is based on the concatenated sequences of seven *C. concisus* housekeeping genes. Each sample is annotated according to sampling site (dot=gut mucosal biopsy, triangle=saliva, diamond=faeces). Colours represent clinical presentation (red=IBD, yellow=GE, green=HC, black=reference strains). Text=BIGSdb reference and isolate ID's (more information regarding this can be found in Table S1). Reference strain *C. curvus* 525-92 is used as an outgroup.

Supplementary table 1: Overview of genes matching COG class description according to GS.

COG class description	Number of corresponding genes in the GS I core genome	Number of corresponding genes in the GS II core genome	Difference in proportion of respective core genomes (%)
Amino acid transport and metabolism	75	127	2,323%
Carbohydrate transport and metabolism	43	82	2,130%
Cell cycle control, cell division, chromosome partitioning	13	27	0,836%
Cell motility	38	56	0,452%
Cell wall/membrane/envelope biogenesis	46	65	0,305%
Coenzyme transport and metabolism	18	27	0,255%
Defense mechanisms	12	18	0,170%
Energy production and conversion	21	30	0,167%
Function unknown	10	15	0,142%
General function prediction only	6	9	0,085%
Inorganic ion transport and metabolism	66	88	-0,020%
Intracellular trafficking, secretion, and vesicular transport	31	39	-0,212%
Lipid transport and metabolism	14	16	-0,236%
Multiple classes	76	98	-0,313%
Nucleotide transport and metabolism	44	55	-0,332%
Posttranslational modification, protein turnover, chaperones	17	18	-0,411%
Replication, recombination and repair	80	102	-0,430%
Secondary metabolites biosynthesis, transport and catabolism	24	25	-0,615%
Signal transduction mechanisms	74	84	-1,297%
Transcription	49	50	-1,347%
Translation, ribosomal structure and biogenesis	104	120	-1,653%
Total number of genes assigned to COG	861	1151	
Total number of genes in respective core genomes	975	1367	

Supplementary Table 3: CRISPR elements and *C.conciscus* plasmid integration genes for 141 *C. conciscus* genomes, and distribution in clinical groups

	CRISPR-associated genes																			
	<i>cas1_1</i>	<i>cas1_2</i>	<i>cas1_3</i>	<i>cas1_4</i>	<i>cas1_5</i>	<i>cas1_6</i>	<i>cas1_7</i>	<i>cas1_8</i>	<i>cas1_9</i>	<i>cas2</i>	<i>cas2_1</i>	<i>cas2_2</i>	<i>cas3</i>	<i>cas9</i>	<i>CAS10/Csm1</i>	<i>csd</i>	<i>csd_1</i>	<i>csd_2</i>	<i>csmA</i>	<i>csm3</i>
Genomospecies I (n=40)	0	0	10	0	0	0	0	0	0	0	10	0	0	0	10	25	1	40	0	10
Genomospecies II (n=101)	44	1	19	1	42	1	1	1	1	82	19	1	69	1	21	60	0	34	1	21
Crohn's disease (n=28)	4	0	10	0	9	0	0	1	0	13	10	0	11	0	10	15	0	15	0	10
Gastroenteritis (n=7)	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	5	0	3	0	0
Healthy Controls (37)	13	0	9	0	11	1	0	0	0	23	9	0	19	0	10	21	1	24	0	10
Ulcerative colitis (n=19)	7	1	5	1	6	0	1	0	0	12	5	1	12	1	6	13	0	10	1	6
UC-IPAA surgery (n=40)	16	0	5	0	14	0	0	0	0	28	5	0	24	0	5	24	0	19	0	5
unknown (n=9)	4	0	0	0	1	0	0	0	1	5	0	0	2	0	0	7	0	3	0	0

	<i>Campylobacter conciscus</i> plasmid integration island genes																		
	<i>virB2</i>	<i>virB2_2</i>	<i>virB4</i>	<i>virB4_2</i>	<i>virB4_3</i>	<i>virB5</i>	<i>virB8</i>	<i>virB8_2</i>	<i>virB8_3</i>	<i>virB9</i>	<i>virB9_2</i>	<i>virB9_3</i>	<i>VirB10</i>	<i>VirB10_2</i>	<i>VirB10_3</i>	<i>VirB11</i>	<i>VirB11_2</i>	<i>VirB11_3</i>	<i>VirB11_4</i>
Genomospecies I (n=40)	1	4	0	1	4	1	0	1	5	0	1	5	5	0	1	7	5	0	1
Genomospecies II (n=101)	3	2	2	3	12	3	2	3	13	2	3	12	12	2	3	10	12	2	3
Crohn's disease (n=28)	3	5	0	3	8	3	0	3	9	0	3	9	9	0	3	1	9	0	3
Gastroenteritis (n=7)	1	1	0	1	0	1	0	1	1	0	1	0	0	0	1	0	0	0	1
Healthy Controls (37)	0	0	0	0	4	0	0	0	4	0	0	4	4	0	0	1	4	0	0
Ulcerative colitis (n=19)	0	0	2	0	0	0	2	0	0	2	0	0	0	2	0	4	0	2	0
UC-IPAA surgery (n=40)	0	0	0	0	4	0	0	0	4	0	0	4	4	0	0	9	4	0	0
unknown (n=9)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0

Supplementary table 4:

Overview of isolates, grouped by individual patients. The genetic relatedness of these isolates is depicted in Supplementary figure 1.

Patient ID	Group	Age	Sex	Isolate ID	Source	Location (of biopsy)
3UC	UC	44	M	AAUH-3UCce	Biopsy	Cecum
				AAUH-3UCce2	Biopsy	Cecum 2
4UC	UC	33	F	AAUH-4UCti	Biopsy	Terminal ileum
				AAUH-4UCti-a	Biopsy	Terminal ileum
5CD	CD	24	M	AAUH-5CDo	Saliva	
7UC	IPAA	51	F	AAUH-7UCil	Biopsy	Ileum
8UC	IPAA	41	M	AAUH-8UCo	Saliva	
				AAUH-8UCpp	Biopsy	Proximal pouch
				AAUH-8UCpp-a	Biopsy	Proximal pouch
9UC	IPAA	45	M	AAUH-9UCpp	Biopsy	Proximal pouch
				AAUH-9UCdp	Biopsy	Distal pouch
10UC	IPAA	46	M	AAUH-10UCf2	Faeces	
				AAUH-10UCil-a	Biopsy	Ileum
11UC	UC	63	F	AAUH-11UCco	Saliva	
				AAUH-11UCdes-a	Biopsy	Descending colon
				AAUH-11UCsig-a	Biopsy	Sigmoideum
12CD	CD	63	F	AAUH-12CDo	Saliva	
				AAUH-12CDti2-a	Biopsy	Terminal ileum 2
				AAUH-12CDti4-a	Biopsy	Terminal ileum 4
				AAUH-12CDti5-a	Biopsy	Terminal ileum 5
				AAUH-12CDce	Biopsy	Cecum
				AAUH-12CDtra-a	Biopsy	Transverse colon
				AAUH-12CDtra2-a	Biopsy	Transverse colon 2
				AAUH-12CDdes2	Biopsy	Descending colon 2
				AAUH-12CDdes3	Biopsy	Descending colon 3
				AAUH-12CDdes4	Biopsy	Descending colon 4
				AAUH-12CDsig	Biopsy	Sigmoideum
AAUH-12CDrec-a	Biopsy	Rectum				
15UC	IPAA	28	M	AAUH-15UCpp	Biopsy	Proximal pouch
				AAUH-15UCdp	Biopsy	Distal pouch
				AAUH-15UCdp-a	Biopsy	Distal pouch
16UC	IPAA	48	F	AAUH-16UCo-a	Saliva	
				AAUH-16UCdp	Biopsy	Distal pouch
				AAUH-16UCdp3	Biopsy	Distal pouch 3
				AAUH-16UCdp5	Biopsy	Distal pouch 5
				AAUH-16UCf	Faeces	
				AAUH-16UCf2	Faeces	
AAUH-16UCf3	Faeces					
20UC	IPAA	56	F	AAUH-20UCco	Saliva	
				AAUH-20UCf	Faeces	
22UC	IPAA	44	M	AAUH-22UCpp-a	Biopsy	Proximal pouch
35UC	IPAA	23	F	AAUH-35UCil-a	Biopsy	Ileum
				AAUH-35UCil2-a	Biopsy	Ileum 2
				AAUH-35UCil3-a	Biopsy	Ileum 3
				AAUH-35UCil4-a	Biopsy	Ileum 4

				AAUH-35UCpp	Biopsy	Proximal pouch
				AAUH-35UCdp	Biopsy	Distal pouch
				AAUH-35UCf	Faeces	
37UC	IPAA	49	M	AAUH-37UCo-a	Saliva	
				AAUH-37UCf	Faeces	
39CD	CD	30	M	AAUH-39CDti-a	Biopsy	Terminal ileum
				AAUH-39CDrec-a	Biopsy	Rectum
				AAUH-39CDf	Faeces	
40UC	UC	51	M	AAUH-40UCf	Faeces	
43UC	UC	67	F	AAUH-43UCce-a	Biopsy	Cecum
				AAUH-43UCf	Faeces	
44UC	UC	36	M	AAUH-44UCsig-a	Biopsy	Sigmoideum
				AAUH-44UCsig6	Biopsy	Sigmoideum 6
47UC	IPAA	38	F	AAUH-47UCil	Biopsy	Ileum
				AAUH-47UCil-a	Biopsy	Ileum
48UC	IPAA	21	M	AAUH-48UCo-a	Saliva	
				AAUH-48UCil-a	Biopsy	Ileum
				AAUH-48UCdp-a	Biopsy	Distal pouch
49UC	IPAA	38	F	AAUH-49UCil-a	Biopsy	Ileum
				AAUH-49UCpp-a	Biopsy	Proximal pouch
				AAUH-49UCf	Faeces	Faeces
51UC	IPAA	41	M	AAUH-51UCf	Faeces	
55UC	UC	57	F	AAUH-55UCtra-a	Biopsy	Transverse colon
58UC	UC	68	F	AAUH-58UCo	Saliva	
59UC	IPAA	42	M	AAUH-59UCpp-a	Biopsy	Proximal pouch
2HC	HC	73	M	AAUH-2HCtra	Biopsy	Transverse colon
3HC	HC	44	M	AAUH-3HCo	Saliva	
				AAUH-3HCce2	Biopsy	Cecum 2
6HC	HC	66	M	AAUH-6HCo-a	Saliva	
8HC	HC	45	F	AAUH-8HCo	Saliva	
				AAUH-8HCo-a	Saliva	
9HC	HC	57	M	AAUH-9HCce	Biopsy	Cecum
				AAUH-9HCasc	Biopsy	Ascending colon
10HC	HC	63	F	AAUH-10HCce	Biopsy	Cecum
				AAUH-10HCdes	Biopsy	Descending colon
				AAUH-10HCdes2	Biopsy	Descending colon 2
				AAUH-10HCdes3	Biopsy	Descending colon 3
				AAUH-10HCdes4	Biopsy	Descending colon 4
				AAUH-10HCdes5	Biopsy	Descending colon 5
				AAUH-10HCdes6	Biopsy	Descending colon 6
				AAUH-10HCdes7	Biopsy	Descending colon 7
				AAUH-10HCtra	Biopsy	Transverse colon
11HC	HC	66	F	AAUH-11HCf	Faeces	
				AAUH-11HCo-a	Saliva	
12HC	HC	56	M	AAUH-12HCf	Faeces	
14HC	HC	69	M	AAUH-14HCce	Biopsy	Cecum
15HC	HC	53	M	AAUH-15HCti	Biopsy	Terminal ileum
19HC	HC	67	F	AAUH-19HCf	Faeces	
				AAUH-19HCf2	Faeces	
20HC	HC	49	F	AAUH-20HCasc	Biopsy	
				AAUH-20HCsig-a	Biopsy	Sigmoideum

				AAUH-20HCrec-a	Biopsy	Rectum
1D	D	57	F	AAUH-1Dce	Biopsy	Cecum
				AAUH-1Dasc	Biopsy	Ascending colon
				AAUH-1Dtra	Biopsy	Transverse colon
				AAUH-2012179281	Faeces	*
2010376221	D	65	M	AAUH-2010376221	Faeces	
2D	D	20	F	AAUH-25Df	Faeces	
				AAUH-25Df3	Faeces	**
Ref.	HC			ATCC 51561	Faeces	
Ref.	CD	12	M	UNSW3	Biopsy	
Ref.	D			UNSW1	Faeces	
Ref.	CD			UNSWCD	Biopsy	
Ref.	D			UNSWCS	Faeces	
Ref.	D			ATCC 51562	Faeces	
Ref.	CD	3	M	UNSW2	Biopsy	
Ref.	D			13826	Faeces	
Ref.				ATCC 33237	Saliva	

Abbreviations: CD: Crohn's disease, UC: Ulcerative colitis, IPAA: J-pouch, HC: healthy control, D:Diarrhea. Isolate ID's indicate patient and isolate source. Isolates ending in -a indicate derivation from cultivation in anaerobic atmosphere, all others derive from microaerobic incubation. Ref.: reference isolates from ncbi.gov. Individual isolates from the same location (i.e. same agarplate) are numerated accordingly. Isolates with only numerical IDs derive from previous studies on patients with persistent diarrhea.* Isolate from faeces taken 4 years prior to isolates from the current study **Isolate derived from different faecal sample taken three months after initial faecal sample and after two weeks treatment with ciprofloxacin.

Supplementary table 5:

Overview of genome assembly information

	Isolate ID	Disease	Source	Genome size (Mbp)	Contigs	N50	GC content (%)
1	AAUH-3UCce	UC	Biopsy	1.93	44	103045	39.49
2	AAUH-3UCce2	UC	Biopsy	1.93	38	101107	39.50
3	AAUH-4UCti	UC	Biopsy	1.98	54	97028	39.26
4	AAUH-4UCti-a	UC	Biopsy	1.98	51	97971	39.20
5	AAUH-5CDo	CD	Saliva	2.10	183	89584	37.26
6	AAUH-7UCil	IPAA	Biopsy	1.98	58	95686	39.44
7	AAUH-8UCo	IPAA	Saliva	1.99	41	154956	39.48
8	AAUH-8UCpp	IPAA	Biopsy	2.02	3	82883	39.46
9	AAUH-8UCpp-a	IPAA	Biopsy	1.87	92	39071	39.66
10	AAUH-9UCpp	IPAA	Biopsy	1.82	47	79679	39.64
11	AAUH-9UCdp	IPAA	Biopsy	2.00	102	59846	39.46
12	AAUH-10UCf2	IPAA	Faeces	1.99	36	180736	39.59
13	AAUH-10UCil-a	IPAA	Biopsy	2.08	103	180889	39.41
14	AAUH-11UCo	UC	Saliva	1.89	112	161715	37.46
15	AAUH-11UCdes-a	UC	Biopsy	1.91	51	58626	37.30
16	AAUH-11UCsig-a	UC	Biopsy	1.99	31	159029	39.48
17	AAUH-12CDo	CD	Saliva	1.81	29	197548	37.56
18	AAUH-12CDti2-a	CD	Biopsy	1.81	9	934037	37.56
19	AAUH-12CDti4-a	CD	Biopsy	1.81	34	83693	37.57
20	AAUH-12CDti5-a	CD	Biopsy	1.82	29	187882	37.59
21	AAUH-12CDce	CD	Biopsy	1.86	31	483873	37.46
22	AAUH-12CDtra-a	CD	Biopsy	1.97	58	63299	39.42
23	AAUH-12CDtra2-a	CD	Biopsy	1.81	24	266692	37.59
24	AAUH-12CDdes2	CD	Biopsy	1.85	49	158794	37.53
25	AAUH-12CDdes3	CD	Biopsy	1.81	54	77176	37.64
26	AAUH-12CDdes4	CD	Biopsy	1.85	26	158715	37.46
27	AAUH-12CDsig	CD	Biopsy	1.90	20	257808	37.34
28	AAUH-12CDrec-a	CD	Biopsy	1.91	52	102065	39.63
29	AAUH-15UCpp	IPAA	Biopsy	1.90	36	162832	39.66
30	AAUH-15UCdp	IPAA	Biopsy	2.06	57	52907	39.47
31	AAUH-15UCdp-a	IPAA	Biopsy	2.03	130	35098	39.51
32	AAUH-16UCo-a	IPAA	Saliva	1.88	43	151248	37.68
33	AAUH-16UCdp	IPAA	Biopsy	1.92	57	162832	39.69
34	AAUH-16UCdp3	IPAA	Biopsy	1.99	66	64884	39.67
35	AAUH-16UCdp5	IPAA	Biopsy	2.04	39	142542	39.48
36	AAUH-16UCf	IPAA	Faeces	1.89	33	392237	37.68
37	AAUH-16UCf2	IPAA	Faeces	1.88	61	64374	37.67
38	AAUH-16UCf3	IPAA	Faeces	1.99	66	64884	39.67
39	AAUH-20UCo	IPAA	Saliva	1.81	24	235464	37.58
40	AAUH-20UCf	IPAA	Faeces	1.91	81	45189	39.70
41	AAUH-22UCpp-a	IPAA	Biopsy	2.02	44	131008	39.58
42	AAUH-35UCil-a	IPAA	Biopsy	1.93	55	97085	39.54
43	AAUH-35UCil2-a	IPAA	Biopsy	1.93	41	138370	39.52
44	AAUH-35UCil3-a	IPAA	Biopsy	1.93	39	97130	39.52
45	AAUH-35UCil4-a	IPAA	Biopsy	1.94	61	80285	39.49
46	AAUH-35UCpp	IPAA	Biopsy	1.93	40	134251	39.47
47	AAUH-35UCdp	IPAA	Biopsy	1.96	70	138943	39.55
48	AAUH-35UCf	IPAA	Faeces	1.97	76	87016	39.52
49	AAUH-37UCo-a	IPAA	Saliva	1.90	41	89957	37.49
50	AAUH-37UCf	IPAA	Faeces	2.00	54	87543	39.52

51	AAUH-39CDti-a	CD	Biopsy	1.93	62	120353	39.47
52	AAUH-39CDrec-a	UCD	Biopsy	2.01	94	42323	39.38
53	AAUH-39CDf	CD	Faeces	1.96	54	136855	39.51
54	AAUH-40UCf	UC	Faeces	2.11	178	34887	39.42
55	AAUH-43UCce-a	UC	Biopsy	2.01	118	73289	39.76
56	AAUH-43UCf	UC	Faeces	1.97	67	62023	39.64
57	AAUH-44UCsig-a	UC	Biopsy	2.05	111	54029	39.40
58	AAUH-44UCsig6	UC	Biopsy	2.02	235	16737	39.56
59	AAUH-47UCil	IPAA	Biopsy	1.94	69	70435	39.78
60	AAUH-47UCil-a	IPAA	Biopsy	1.92	90	42174	39.81
61	AAUH-48UCo-a	IPAA	Saliva	1.88	80	45066	37.58
62	AAUH-48UCil-a	IPAA	Biopsy	1.97	58	65623	39.70
63	AAUH-48UCdp-a	IPAA	Biopsy	1.94	202	19218	39.78
64	AAUH-49UCil-a	IPAA	Biopsy	2.12	160	43381	39.27
65	AAUH-49UCpp-a	IPAA	Biopsy	2.1	162	52807	39.59
66	AAUH-49UCf	IPAA	Faeces	2.22	118	97148	39.15
67	AAUH-51UCf	IPAA	Faeces	1.9	61	100202	37.29
68	AAUH-55UCtra-a	UC	Biopsy	1.88	88	37875	37.43
69	AAUH-58UCo	UC	Saliva	2.02	105	34563	39.47
70	AAUH-59UCpp-a	IPAA	Biopsy	1.92	49	102305	39.62
71	AAUH-2HCtra	HC	Biopsy	1.99	84	52482	39.42
72	AAUH-3HCo	HC	Saliva	2.03	336	26870	37.93
73	AAUH-3HCce2	HC	Biopsy	2.07	328	33756	39.88
74	AAUH-6HCo-a	HC	Saliva	1.78	85	32468	37.59
75	AAUH-8HCo	HC	Saliva	1.78	146	23267	37.76
76	AAUH-8HCo-a	HC	Saliva	1.81	132	25221	37.64
77	AAUH-9HCce	HC	Biopsy	1.98	189	21463	39.71
78	AAUH-9HCasc	HC	Biopsy	1.80	86	41150	37.68
79	AAUH-10HCce	HC	Biopsy	1.94	84	43541	39.61
80	AAUH-10HCdes	HC	Biopsy	1.91	55	65110	39.58
81	AAUH-10HCdes2	HC	Biopsy	1.90	79	59463	39.62
82	AAUH-10HCdes3	HC	Biopsy	1.91	59	74769	39.59
83	AAUH-10HCdes4	HC	Biopsy	1.90	90	45360	39.64
84	AAUH-10HCdes5	HC	Biopsy	1.91	84	39333	39.60
85	AAUH-10HCdes6	HC	Biopsy	1.98	102	36601	39.63
86	AAUH-10HCdes7	HC	Biopsy	1.90	74	50724	39.62
87	AAUH-10HCtra	HC	Biopsy	1.95	72	60348	39.59
88	AAUH-11HCf	HC	Faeces	1.92	229	14154	37.71
89	AAUH-11HCo-a	HC	Saliva	1.93	218	14905	37.70
90	AAUH-12HCf	HC	Faeces	1.96	161	22427	39.49
91	AAUH-14HCce	HC	Biopsy	1.83	157	21790	37.66
92	AAUH-15HCti	HC	Biopsy	1.95	129	24790	39.84
93	AAUH-19HCf	HC	Faeces	2.01	85	48338	39.51
94	AAUH-19HCf2	HC	Faeces	1.98	155	22062	39.64
95	AAUH-20HCasc	HC	Biopsy	2.04	319	20031	39.81
96	AAUH-20HCsig-a	HC	Biopsy	2.04	152	33006	39.43
97	AAUH-20HCrec-a	HC	Biopsy	2.00	82	84768	39.31
98	AAUH-1Dce	D	Biopsy	2.2	356	48282	39.56
99	AAUH-1Dasc	D	Biopsy	1.88	234	13858	39.77
100	AAUH-1Dtra	D	Biopsy	1.91	93	39458	39.56
101	AAUH-2012179281	D	Faeces	1.92	34	138913	37.35
102	AAUH-2010376221	D	Faeces	1.85	84	48012	37.67
103	AAUH-25Df	D	Faeces	1.81	31	134513	39.74
104	AAUH-25Df3	D	Faeces	2.01	51	121167	39.38
	ATCC 51561						

	UNSW3 (ref)						
	UNSW1 (ref)						
	UNSWCD (ref)						
	UNSWCS (ref)						
	ATCC 51562 (ref)						
	UNSW2 (ref)						
	13826 (BAA-1457) (ref)						
	ATCC 33237 (ref)						

Table 2: Genome assembly information and NCBI accession numbers. CD: Crohn's disease, UC: Ulcerative colitis, IPAA: Ileal-pouch-anal-anastomosis (J-pouch), HC: healthy control