

METAGENOMIC APPROACH OF GUT MICROBIOTA FROM CHRONIC KIDNEY DISEASE RATS

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BACKGROUND:

Gut microbiota is known to function in producing uremic toxins (UTs) and their precursors, such as indoles, phenols and so on. We have reported that UTs production may be not only the cause of uremic symptoms but also correlate with a subset of indigenous gut microbes and affects the composition of gut microbiota in chronic kidney disease (CKD) model rats. To elucidate the role of gut microbiota in CKD rats, we performed the metagenomic analysis of gut microbiota in normal rats and CKD model rats. AST-120, a spherical carbon adsorbent of intestinal small-molecular-weight UTs and/or their precursors, was used to evaluate the effects of UTs on gut microbiota.

METHODS:

The collection of feces samples were carried in normal rats and rats subjected to 5/6 renal removal. AST-120 was administered as chow mixed with 8% AST-120.

Gut microbiota from feces at 24 weeks after baseline were analyzed using 454-pyrosequencing of a hypervariable V1-V2 region of the 16S rRNA gene in combination with barcode sequences. Operational taxonomic unit clustering and UniFrac analysis were performed. Relative abundances were calculated from taxonomic assignment.

Metagenomic analysis of gut microbiota from feces at 24 weeks after baseline was carried out. Functional assignment were performed according to the best-hit pairs in the BLASTP analysis against Kyoto Encyclopedia of Genes and Genomes (KEGG) GENES database.

RESULTS:

(1) 16S rRNA analysis

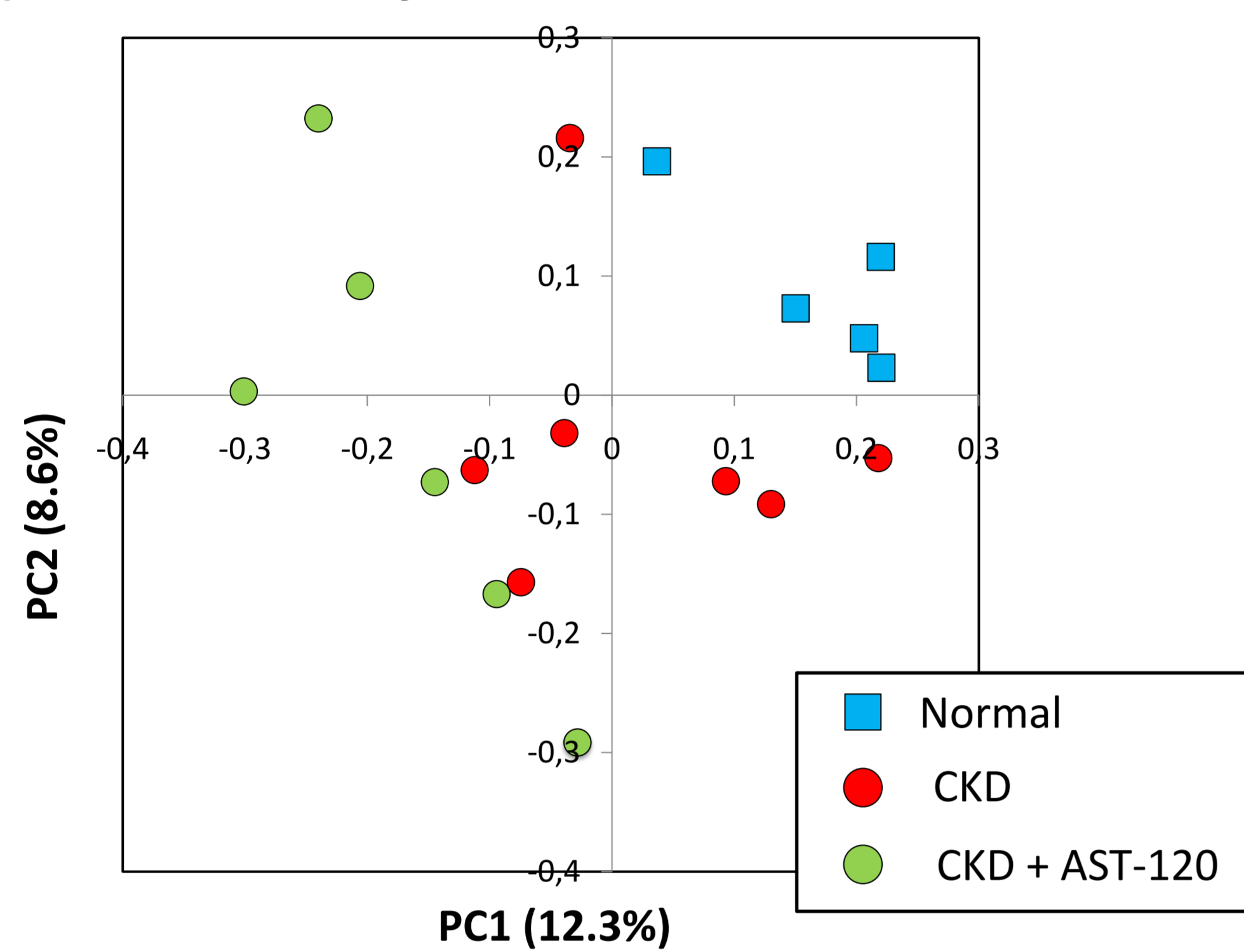


Fig.1 PCoA plot of UniFrac (Similarity of gut microbiota)

(2) Metagenomic analysis

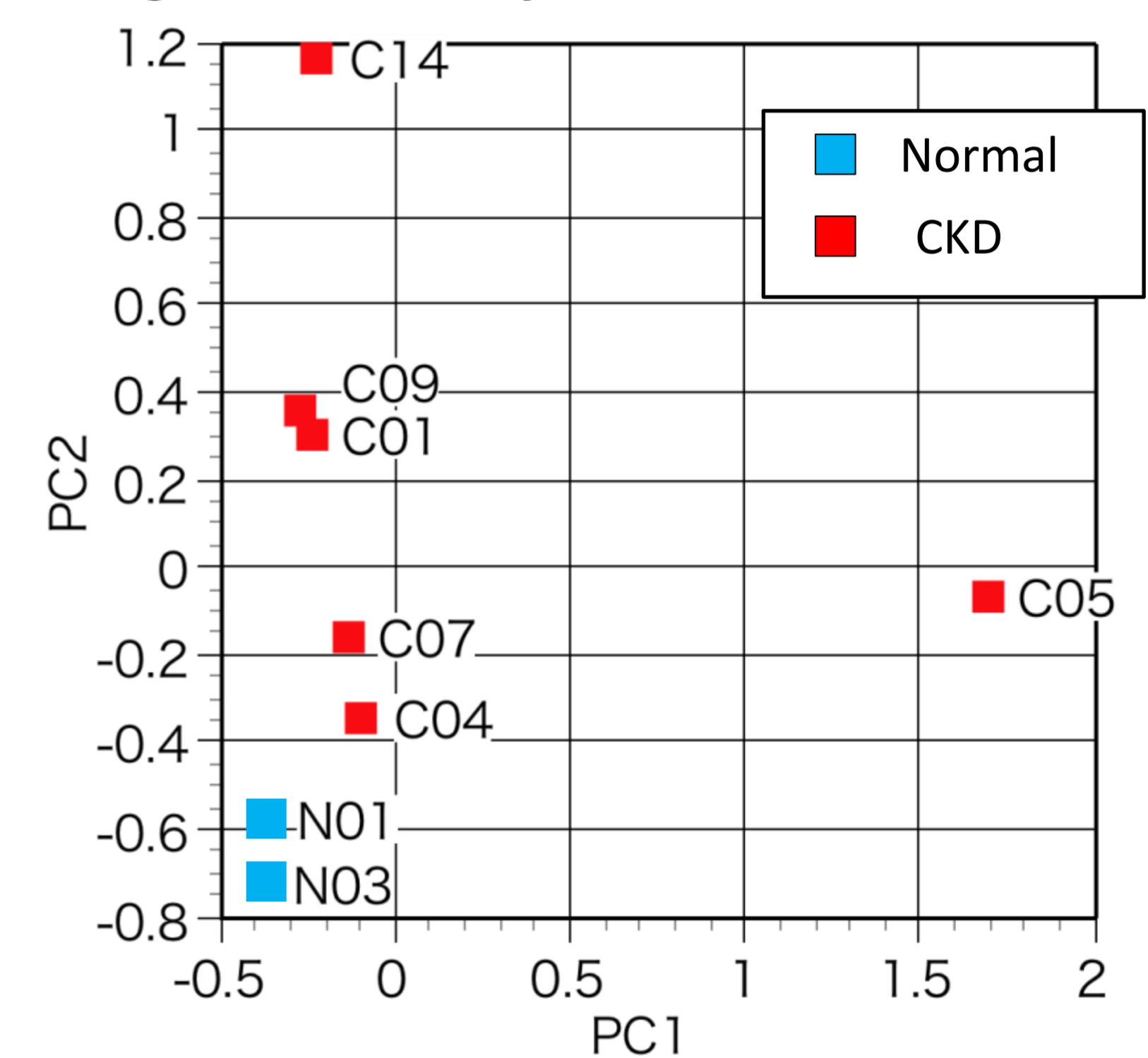


Fig.2 BLASTP based distance matrix (Similarity of gut microbiota)

Table 2 OTUs increased in CKD rats and decreased in AST-120 administration

OTU name	Assignment			Relative abundance (%)			Relative ratio		
	Phylum	Species	Identity (%)	Normal	CKD	CKD+AT-120	CKD/Normal	CKD+AST-120	
OTU01356	Actinobacteria	<i>Eggerthella sinensis</i>	84.7	0.01	0.04	0.00	3.2	-	
OTU01335		<i>[Clostridium] lituseburensis</i>	84.0	0.01	0.14	0.01	10.7	25.7	
OTU00481		<i>Clostridium herbivorans</i>	89.3	0.02	0.14	0.04	6.9	3.6	
OTU00231		<i>Clostridium methylpentosum</i>		82.5	0.09	0.33	0.03	3.6	10.0
OTU00049				78.9	0.10	0.20	0.08	2.0	2.5
OTU00491		<i>Clostridium saccharogumia</i>	83.1	0.01	0.04	0.00	2.9	-	
OTU00115		<i>Clostridium sp. 7_3_54FAA</i>	89.0	0.03	0.16	0.08	4.7	2.0	
OTU00425		<i>Clostridium sp. A9</i>		94.1	0.39	1.11	0.14	2.8	8.0
OTU00016				95.3	0.05	0.42	0.03	7.9	12.7
OTU01097		<i>Clostridium sp. C01-2409</i>		93.3	0.02	0.19	0.02	9.3	11.1
OTU00221				96.3	0.01	0.03	0.00	2.1	-
OTU00640		<i>Clostridium sp. Culture Jar-13</i>	91.4	0.07	0.15	0.07	2.1	2.1	
OTU00180		<i>Clostridium sp. Culture-23</i>	96.0	0.08	0.28	0.13	3.5	2.1	
OTU00991		<i>Clostridium sp. HGF2</i>	87.9	0.01	0.13	0.06	10.0	2.4	
OTU00028		<i>Clostridium sp. strain Z6</i>	80.9	0.01	0.22	0.03	16.4	7.9	
OTU00617		<i>Clostridium symbiosum</i>	92.0	0.03	0.07	0.03	2.0	2.0	
OTU01032		<i>Clostridium xylanovorans</i>	84.5	0.03	0.10	0.01	3.9	9.4	
OTU01006		<i>Coprococcus comes</i>	93.8	0.02	0.07	0.02	3.3	4.0	
OTU00410		<i>Desulfotomaculum sp. GY-2</i>	82.7	0.02	0.16	0.03	7.9	5.7	
OTU00482		<i>Dorea formicigenerans</i>	94.4	0.03	0.18	0.06	6.8	3.0	
OTU00209		<i>Eubacterium coprostanoligenes</i>	88.6	0.03	0.05	0.02	2.0	2.4	
OTU00510		<i>Eubacterium desmolans</i>	84.3	1.75	4.74	0.14	2.7	34.1	
OTU00459		<i>Eubacterium oxidoreducens</i>	91.7	0.22	0.61	0.00	2.8	-	
OTU00583	<i>Eubacterium plexicaudatum</i>	90.5	0.06	0.25	0.09	4.2	2.7		
OTU00097	<i>Eubacterium rangiferina</i>	85.7	0.11	0.34	0.02	3.2	15.4		
OTU00621	Firmicutes		84.8	0.03	0.54	0.09	16.1	5.7	
OTU00390			84.2	0.03	0.46	0.01	17.1	82.3	
OTU00866		<i>Eubacterium siraeum</i>	87.7	0.05	0.12	0.02	2.3	7.4	
OTU00441			79.7	0.01	0.06	0.01	4.3	5.1	
OTU00412		<i>Eubacterium sp. 3_1_31</i>	86.7	0.02	0.07	0.02	3.6	4.3	
OTU00501		<i>Eubacterium sp. Pei061</i>	75.4	0.03	0.69	0.05	20.6	13.7	
OTU00832		<i>Eubacterium xylanophilum</i>	85.7	0.02	0.05	0.01	2.6	4.7	
OTU00148		<i>Faecalibacterium prausnitzii</i>	80.4	0.08	0.22	0.06	2.7	3.6	
OTU00271			92.6	0.03	0.08	0.03	2.3	2.7	
OTU00145		<i>Flavonifractor plautii</i>	92.3	0.01	0.03	0.01	2.1	2.6	
OTU01825	<i>Marvinbryantia formatexigens</i>	88.4	0.02	0.04	0.01	2.1	3.9		
OTU00889		84.4	0.21	0.70	0.25	3.3	2.8		
OTU01619		83.4	0.20	0.65	0.01	3.2	116.6		
OTU00070	<i>Oscillibacter valericigenes</i>		85.6	0.01	0.22	0.00	16.4	-	
OTU00945			81.9	0.04	0.15	0.05	3.7	3.0	
OTU00108			91.1	0.02	0.07	0.02	3.6	4.3	
OTU01135			85.2	0.01	0.05	0.01	3.6	8.6	
OTU01405			91.4	0.01	0.04	0.01	2.9	3.4	
OTU00387		<i>Roseburia hominis</i>	89.3	0.03	0.06	0.00	2.3	-	
OTU00176		<i>Ruminococcus bromii</i>	85.5	0.35	1.26	0.59	3.6	2.1	
OTU00154		<i>Ruminococcus sp. ID1</i>	87.7	0.01	0.04	0.00	2.9	-	
OTU00710		<i>Ruminococcus sp. M-1</i>	92.0	0.05	0.20	0.09	4.3	2.1	
OTU00315		<i>Ruminococcus sp. YE281</i>	78.2	0.02	0.10	0.03	4.8	3.4	
OTU00934	<i>Subdoligranulum sp. 4_3_54A2FAA</i>	78.6	0.01	0.06	0.00	4.3	-		

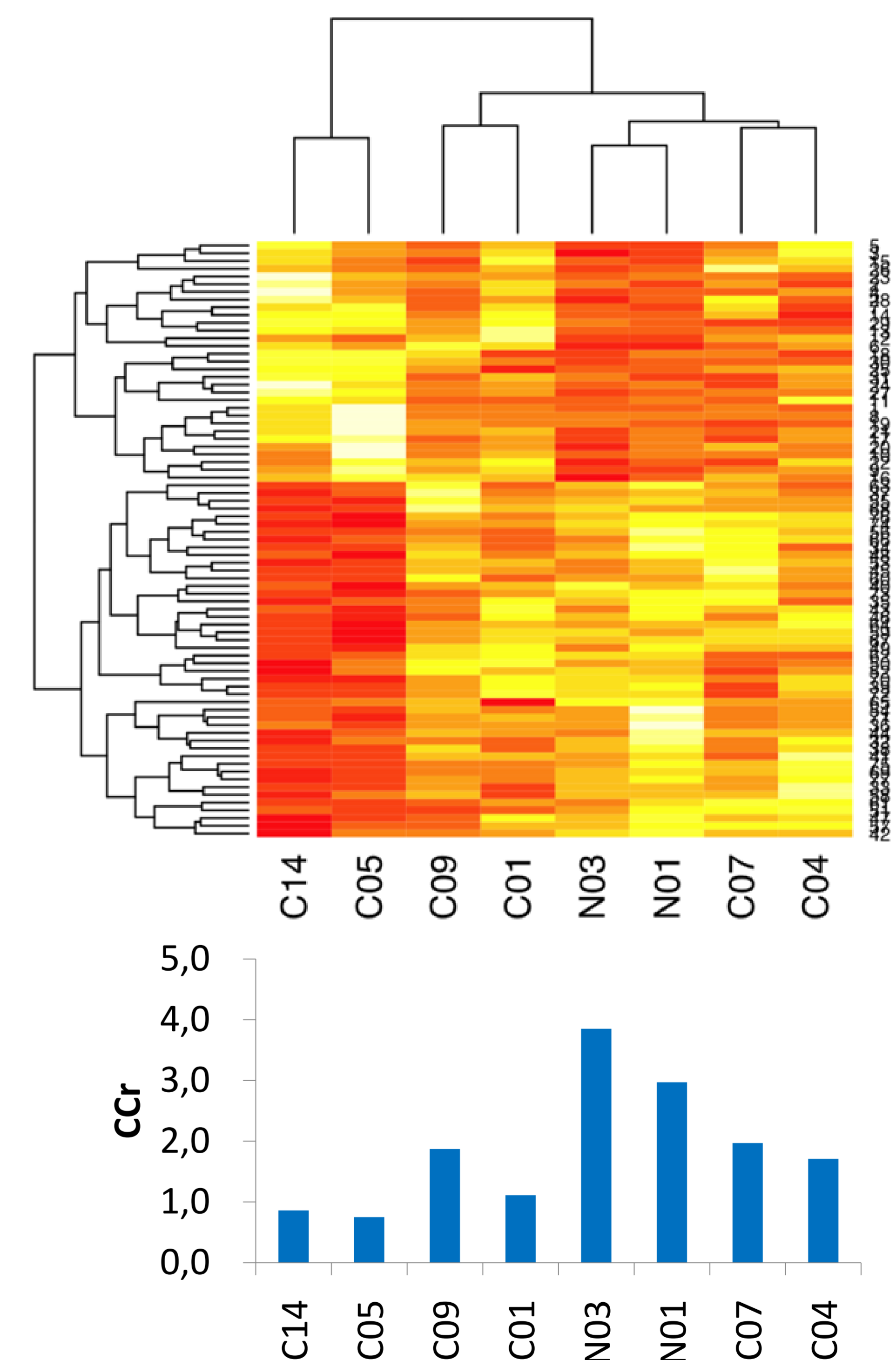


Fig.3 KEGG based heat map and Ccr

CONCLUSIONS:

The data indicated that gut microbiota from normal rats and those from CKD rats formed groups respectively.

(1) 16S rRNA analysis: *Clostridium*, *Eubacterium*, *Oscillibacter* and *Ruminococcus* increased in CKD rats, and they decreased in AST-120 administration.

(2) Metagenomic analysis: Enriched genes in CKD rats included cell motility such as chemotaxis and flagellar assembly related proteins.