

# Effect of *C. difficile* spent medium on fecal microbiota community structure in a simple *in vitro* model

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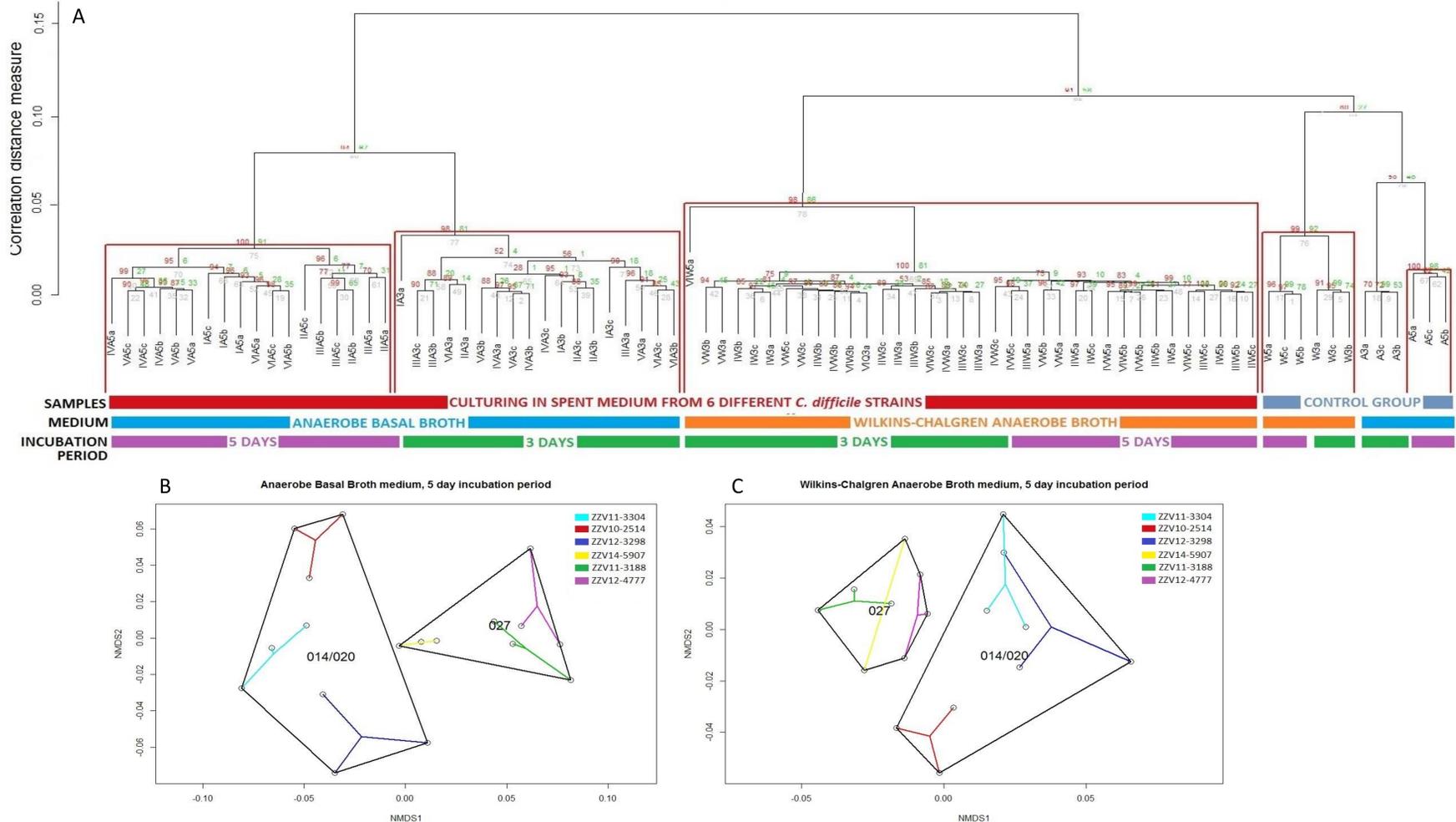


## INTRODUCTION

Crucial for the development of *C. difficile* infection (CDI) is disturbance of the normal gut microbiota, which is usually due to treatment with antibiotics<sup>1</sup>. Individuals colonized with *C. difficile* have lower diversity of gut microbiota, with the general decrease of anaerobic bacterial species and an increased proportion of facultative anaerobes<sup>2</sup>. During colonization *C. difficile* could influence the gut microenvironment, e.g. via excreted metabolites.

To observe the impact of *C. difficile* on growth of common gut bacteria we analyzed the differences in bacterial community structure of fecal microbiota after *in vitro* growth in spent medium from *C. difficile*.

Fig. 1.: (A) Cluster analysis using correlation method as a distance measure and average method (UPGMA) as tree building algorithm (R, pvclust package). Each nod is assessed with two p-values, i.e. Approximately Unbiased (red) and Bootstrap Probability (green). Red squares enframe samples that form clusters with 95 % confidence interval. (B, C) Non-Metric Multidimensional Scaling (NMDS) analysis, done separately for samples cultured in Anaerobe Basal Broth medium (A) and Wilkins-Chalgren Anaerobe Broth medium (B) (R, vegan package). Black lines indicate *C. difficile* ribotypes, whereas colored lines their corresponding strains.



## RESULTS AND DISCUSSION

OTU analysis shows significant differentiation in response to growth media used and incubation duration (AMOVA:  $p = 0,002$ ; HOMOVA:  $p < 0,001$ ; Unifrac Unweighted:  $p < 0,001$ ). When data was subset into four unique treatments according to two growth media and two time points, significant differentiation between ribotypes and control group was noticed (AMOVA:  $p < 0,001$ ) (Fig. 1).

OTUs that were significantly associated with given ribotype and/or medium are presented in Table 1.

Despite distinction between different *C. difficile* ribotypes used to prepare spent medium, analysis of OTUs responsible for this clustering does not show elevated deleterious effect of 027 ribotype in comparison to 014/020 as suggested in previous studies<sup>3, 4, 5</sup>.

## METHODS

Six different *C. difficile* strains, corresponding to two ribotypes (014/020, 027), were incubated for 48 hours in two different culture media (Wilkins Chalgren Anaerobe Broth - WCAB, Anaerobe Basal Broth - ABB).

The spent medium was subsequently used for culturing of fecal microbiota. Samples were taken after 3 and 5 day incubation period and total bacterial DNA was extracted.

Bacterial community composition was determined by paired-end sequencing on Illumina MiSeq platform, targeting V3-V4 hypervariable region of the 16S rRNA gene. Reagents and primers were used as advised by Illumina in 16S Metagenomic Sequencing Library Preparation guide.

Data analysis was performed with Mothur software<sup>6</sup> following MiSeq SOP.

## REFERENCES

- Rupnik M., Wilcox M.H., Gerding D.N. *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. *Nat Rev Microbiol* 2009, 7, 526-536.
- Zalig S, Rupnik M. *Clostridium difficile* infection and gut microbiota. *Semin Colon Rectal Surg* 2014, in press.
- Skraban J, Dzeroski S, Zenko B, Mongus D, Gangl S, Rupnik M. Gut microbiota patterns associated with colonization of different *Clostridium difficile* ribotypes. *PLoS ONE* 2013, 8, e58005.
- Rea MC, O'Sullivan O, Shanahan F, O'Toole PW, Stanton C, Ross RP, et al. *Clostridium difficile* carriage in elderly subjects and associated changes in the intestinal microbiota. *J Clin Microbiol* 2012, 50, 867-875.
- Lawley TD, Clare S, Walker AW, Stares MD, Connor TR, Raisen C, et al. Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing *Clostridium difficile* disease in mice. *PLoS Pathog* 2012, 8, e1002995.
- Schloss P.D., Westcott S.L., Ryabin T., et al. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Appl. Environ. Microbiol.* 2009, 75, 23, 7537-7541.

Table 1.: Genus level classification of operational taxonomic units (OTUs), that significantly ( $p < 0,05$ ) diverge between samples cultured in *C. difficile* spent medium and control samples. Table is separated according to two ribotypes and growth media used. Green color indicates increase while red decrease of the OTU abundance in a given treatment. No indicates number of OTUs that classified into corresponding genus.

	Ribotype 014/020		Ribotype 027	
ABB medium	GenusNo	GenusNo	GenusNo	GenusNo
	Bacteroides3	Sutterella2	Bacteroides3	Clostridium_XIVa3
	Parabacteroides2	Clostridium_XIVa2	Clostridium_XIVa2	Streptococcus2
	Clostridium_XIVa2	Bacteroides2	Barnesiella1	Sutterella2
	Escherichia_Shigella1	Lactobacillus2	Bifidobacteria1	Lactobacillus2
	Phascolarctobacterium1	Veillonella1	Blautia1	Streptococcus1
	Dorea1	Acidaminococcus1	Clostridium_sensu_stricto1	Alistipes1
	Clostridium_XVIII1	Streptococcus1	Clostridium_XVIII1	Dorea1
	Clostridium_sensu_stricto1	Alistipes1	Collinsella1	Faecalibacterium1
	Blautia1	Dorea1	Dorea1	Bacteroides1
Holdemanella1	Faecalibacterium1	Erysipelotrichaceae_incertae_sedis1	Eggerthella1	
Collinsella1	Eggerthella1	Escherichia_Shigella1	Gordonibacter1	
Parasutterella1	Gordonibacter1	Holdemanella1	Odoribacter1	
Lachnospiraceae_incertae_sedis1	Odoribacter1	Lachnospiraceae_incertae_sedis1	Erysipelotrichaceae_incertae_sedis1	
WCAB medium	Bacteroides7	Escherichia_Shigella2	Bacteroides7	Lactobacillus2
	Clostridium_XIVa3	Streptococcus2	Clostridium_XIVa3	Streptococcus2
	Bifidobacteria1	Alistipes1	Blautia1	Alistipes1
	Blautia1	Bacteroides1	Clostridium_XVIII1	Bacteroides1
	Clostridium_sensu_stricto1	Clostridium_sensu_stricto1	Collinsella1	Clostridium_sensu_stricto1
	Collinsella1	Clostridium_XIVa1	Coprococcus1	Clostridium_XIVa1
	Coprococcus1	Coprococcus1	Dorea1	Dorea1
	Dorea1	Dorea1	Eggerthella1	Erysipelotrichaceae_incertae_sedis1
	Eggerthella1	Erysipelotrichaceae_incertae_sedis1	Enterococcus1	Escherichia_Shigella1
	Enterococcus1	Faecalibacterium1	Erysipelotrichaceae_incertae_sedis1	Faecalibacterium1
Erysipelotrichaceae_incertae_sedis1	Lactobacillus1	Flavonifractor1	Gordonibacter1	
Flavonifractor1	Parabacteroides1	Lachnospiraceae_incertae_sedis1	Odoribacter1	
Lachnospiraceae_incertae_sedis1	Parasutterella1	Parabacteroides1	Parabacteroides1	
Parabacteroides1	Roseburia1	Peptostreptococcus1	Parasutterella1	
Phascolarctobacterium1	Veillonella1	Phascolarctobacterium1	Veillonella1	