VARIOUS WAYS OF INTERACTIONS BETWEEN *Clostridium difficile* AND GUT MICROBIOTA

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INTRODUCTION

- *Clostridium difficile* is an intestinal pathogen typically associated with dysbalanced gut microbiota.[1]
- Using a simple *in vitro* batch model we have previously shown that interactions between *C. difficile* and microbiota are bidirectional.[2,3] (Fig.1) *C. difficile* vegetative cells or conditioned media had influenced the diversity and composition of fecal microbiota. Changes in microbiota composition were specific and similar to those observed in patients with *C. difficile* infection (CDI), suggesting that dysbiosis initially caused by e.g. antibiotics and predisposing to CDI, is to some extent maintained by *C. difficile* during and after the infection.
- In the case of microbiota effects on *C. difficile* we have shown that growth is strain dependent, while all strains showed higher sporulation frequency in the co-culture with dysbalanced fecal microbiota.[3]
- Adult dysbalanced microbiota showed different changes as adult healthy microbiota in previous experiments. The aim of our study presented here was to compare the impact of *C. difficile* vegetative cells and *C. difficile* conditioned medium on gut microbiota of infants under 2 years of age in *in vitro* model.

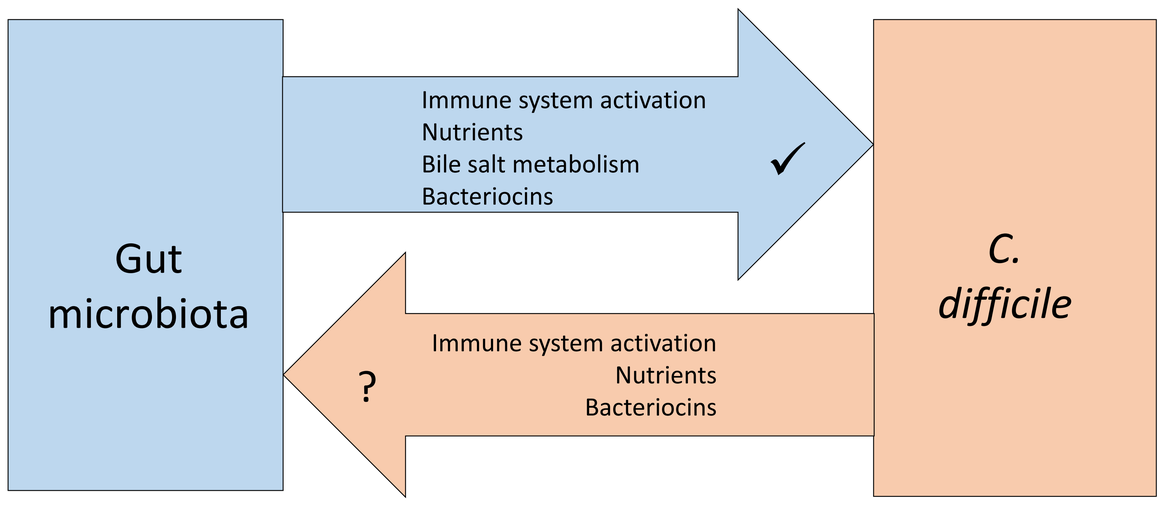

Fig. 1: Possible interactions between gut microbiota and C. difficile.

MATERIALS AND METHODS

Fecal emulsion was prepared by pooling *C. difficile* negative fecal samples of four healthy infants (< 2 years). Six *C. difficile* toxigenic strains, belonging to ribotypes 027 (n=2), 176 (n=2) and 078 (n=2), were selected to prepare spent media for subsequent fecal microbiota culturing. Simultaneously, fecal microbiota was cultured with *C. difficile* vegetative cells (≈ 2 x 10^6 CFU/ml). Samples were taken after 72 hours incubation period and screened for total cell count and spore count of *C. difficile* in co-cultures. After centrifugation pellets were used for total bacterial DNA extraction. Bacterial community composition was determined by paired-end sequencing on Illumina MiSeq platform, targeting V3-V4 hypervariable region of the 16S rRNA gene. MiSeq output data was analysed with statistical tools included in the mothur software (version 1.36.1).

RESULTS

Five out of six tested strains grew significantly better in growth medium only than in co-cultures with infant microbiota (**Fig. 2a**). All six strains formed higher percentage of spores in co-culture with infant microbiota (20 - 57 %), while in control samples spore percentage was lower than 8 %. (**Fig. 2b**).

Cultivation of infant fecal microbiota in the presence of vegetative cells or in the presence of conditioned medium decreased the bacterial diversity (**Fig. 3**) and significant differences were observed for genera within *Bacteroidetes, Firmicutes* and *Proteobacteria* phyla (**Fig. 4**).

CONCLUSIONS

The results indicate that *C. difficile* is able to affect the infant gut microbiota. Changes are similar to those observed in children infected with *C. difficile*.[4] In addition, the high amount of *C. difficile* spores present in co-cultures with microbiota could explain the high rate of asymptomatic carriage observed in infants.[5,6]

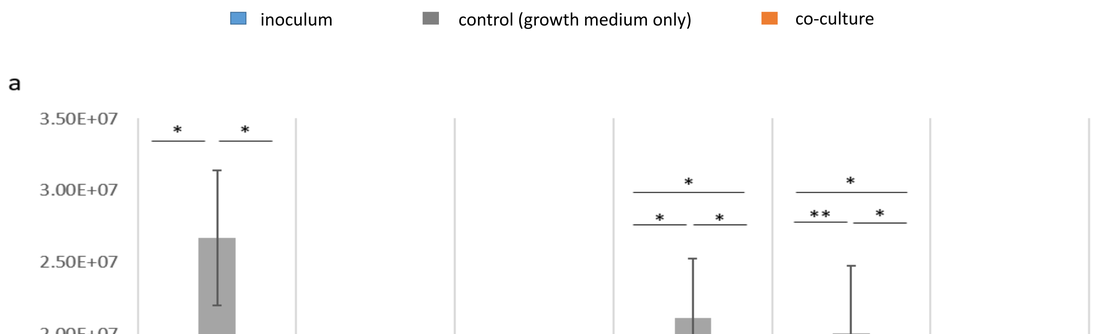

Fig. 2: (a) Total viable cell count with corresponding standard deviation for C. difficile ribotypes 027, 176 and 078 strains*. Total CFU in the inoculum, in the control and in co-culture with infant fecal microbiota is indicated with blue, grey and orange color, respectively. * Significant at p < 0.05. ** Significant at p < 0.01.* ***(b) Percentage of C. difficile spores detected as ethanol resistant CFUs in proportion to total CFUs for ribotypes 027, 176 and 078 strains.*** *Percentage of ethanol resistant CFUs in the control and in combination with infant fecal microbiota is indicated with grey and orange color, respectively.*

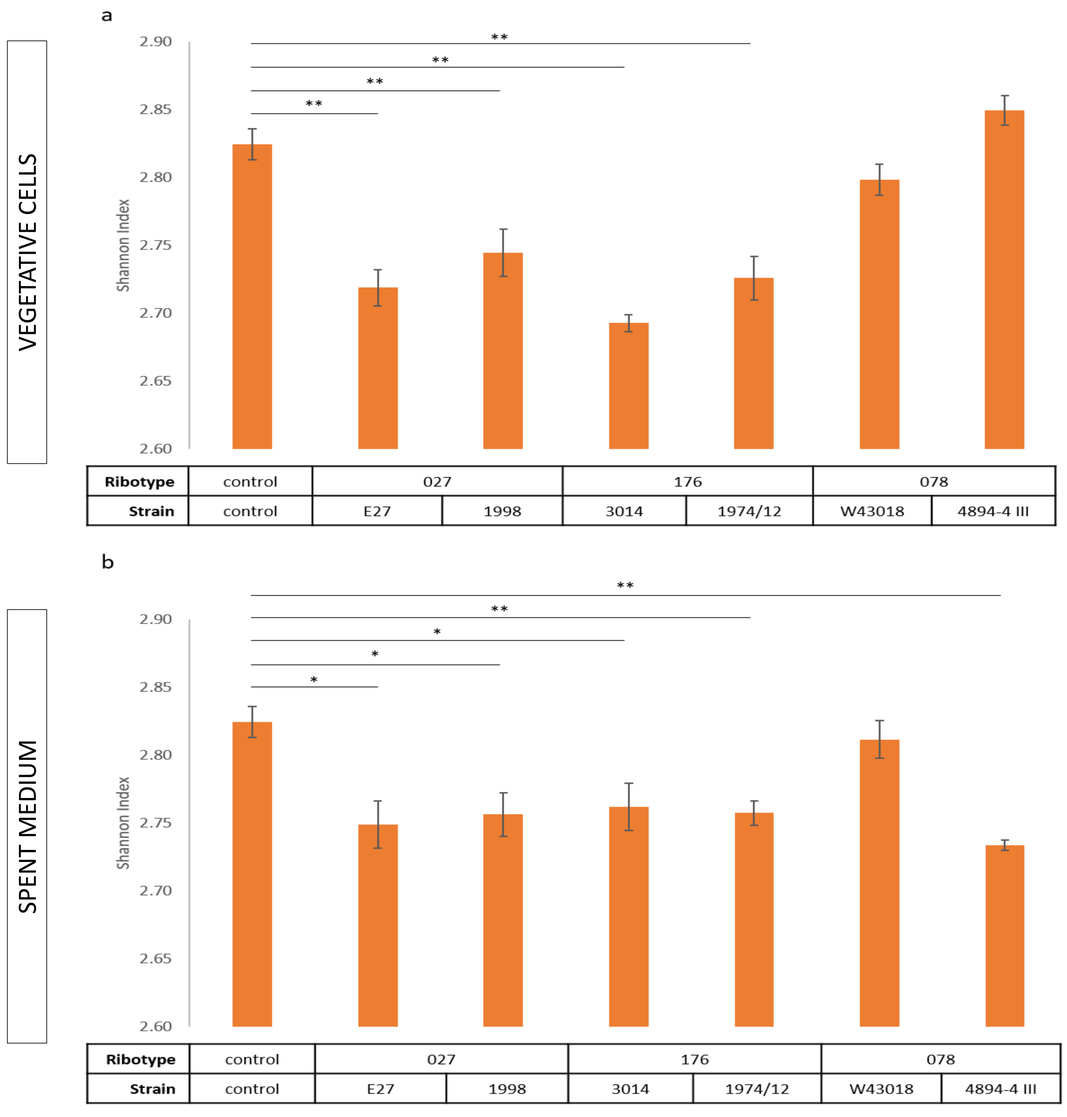

Fig. 3: Plots of Shannon diversity index with corresponding standard deviation for control samples of infant fecal microbiota only and samples of infant fecal microbiota in combination with C. difficile ribotypes 027, 176 and 078 strains (a) or samples of infant fecal microbiota in conditioned media of C. difficile ribotypes 027, 176 and 078 strains (b). ** Significant at p < 0.05. ** Significant at p < 0.01.*

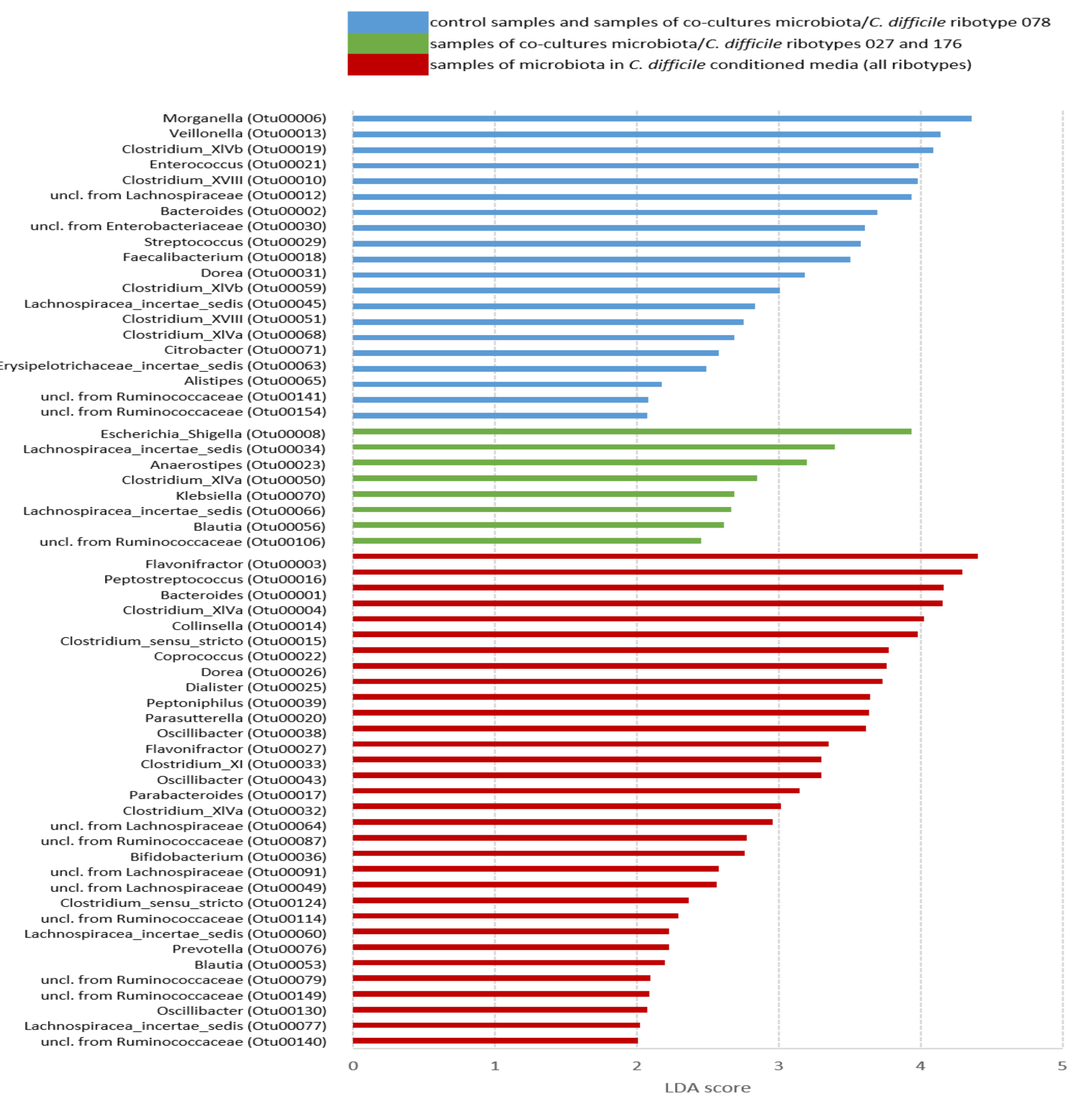

Fig. 4: Differentially represented OTUs in groups of samples. Blue color indicates OTUs significantly associated with control samples of infant fecal microbiota only and samples of co-cultures infant fecal microbiota/*C. difficile* ribotype 078 strains, green color represent samples of co-cultures infant fecal microbiota/*C. difficile* ribotypes 027 and 176 strains and red color corresponds to samples of infant fecal microbiota in *C. difficile* conditioned media (all ribotypes). Presented OTUs were identified by the LEfSe test (mothur software), which uses linear discriminant analysis (LDA) to find OTUs that significantly differ in abundance between all groups of samples.

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