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Analysis of the Intestinal Microbiome of a Recovered *Clostridium difficile* Patient after Fecal Transplantation

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UniversitätsSpital Zürich

Digestion

Original Paper

Digestion 2013;88:243–251
DOI: 10.1159/000355955

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Analysis of Intestinal Microbiome of a Recovered *Clostridium difficile* Patient after Stool Transplantation

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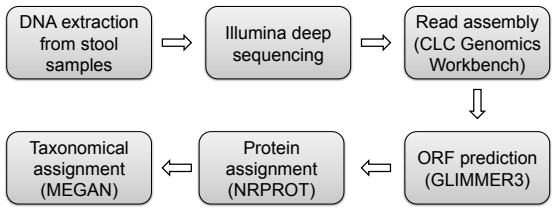
Introduction: Infections with *Clostridium difficile* upon antibiotic disruption of the gut flora lead to intestinal inflammation and, in severe cases, to intoxication and death. Reconstitution of the intestinal flora by fecal transplantation (FT) is a promising approach to treat severe and recurrent cases of *C. difficile*-associated disease (CDAD). Here, we present the case of a patient with recurrent CDAD that received FT. The patient fully recovered and shows no symptoms until today, about three years after FT. **Methods:** Stool specimens of donor and recipient at different time points after FT were analyzed by metagenomic analysis for stool composition and recolonization of *C. difficile*. DNA extracted from stool specimens of the donor and patient (three time points) was subjected to paired-end illumina sequencing. Obtained read sets were assembled using the CLC Genomics Workbench V5 and open reading frames (ORFs) were predicted. Deduced proteins were compared against the NRPROT database and highest ranked hits analyzed with respect to taxonomic assignment using the MEGAN approach. **Results:** Sequencing of the donor sample resulted in 102,523,070 reads, while sequencing of the patient samples provided 103,715,676 (first time after FT), 62,180,598 (second time) and 101,830,352 reads (third time), yielding total contiguous sequence (contig) lengths of 64,959, 68,167, 92,642, and 69,314 megabases (Mb), respectively. ORF prediction by GLIMMER3 provided 68,887, 69,997, 97,197 and 72,235 ORFs, respectively, with a minimal size of 90 bases. Taxonomical assignment in MEGAN indicated that microbiomes of the recipient was a chimeric, healthy and stable microbiome with no indications of *C. difficile* infection and no recurrence during three years follow-up. **Discussion / Conclusion:** Metagenomic analysis with the here reported approach is suitable for analysis of the intestinal flora after FT. Discussion of different evaluation procedures and data management to analyze the composition of the microbiome may be helpful for further studies. Our results demonstrate that FT from a healthy donor can be used to effectively treat recurrent CDAD and presumably other intestinal diseases to restore a normal and diverse gut microbiome and clearance of the pathogenic effect of *C. difficile*. The procedure is simple, cheap, not harmful and was stable over three years with significant increase in quality of life for the patient. The procedure deserves more credit and more intensive analysis for better approval by doctors and authorities for the benefit of patients.

1. Case Presentation

A 51-year old woman was admitted to our hospital with the sixth episode of recurrent CDAD. Standard treatment with oral vancomycin failed to cure the disease. Thus, FT was performed with donor stool from the patient's sister that was tested negative for various pathogens including *C. difficile*. The patient subsequently reported changes in the frequency of bowel movements and intermittent obstipation that both ceased after ten weeks. To date, the patient remains free of any symptoms for now about three years from FT and reports significant amelioration of her general physical condition.

2. Intestinal Metagenomics: Experimental Workflow



3. Illumina Sequencing and Assembly

Sample	Total no. of reads	Total contig length (Mb)	Average (maximal) contig length (bb)	N50 value
Donor, 2010-04-25	102,523,070	64,959	3,390 (305,461)	5,181
Recipient, 2010-11-01	103,715,676	68,167	4,533 (679,591)	9,960
Recipient, 2010-11-12	62,180,598	92,642	5,095 (678,885)	12,113
Recipient, 2010-11-25	101,830,352	69,314	3,991 (482,208)	6,844

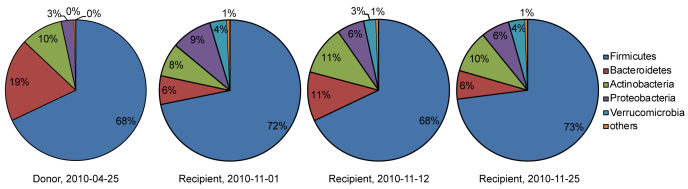
120 basepair paired-end illumina reads were acquired in a multiplex run. The CLC Genomics Workbench (CLC Bio) was used for *de novo* assembly to contigs.

4. ORF Prediction and BLASTP Analysis

Sample	Predicted ORFs	BLASTP against NRPROT (hits / no nits)
Donor, 2010-04-25	68,887	54,673 / 14,214
Recipient, 2010-11-01	69,997	56,352 / 13,645
Recipient, 2010-11-12	97,197	77,714 / 19,483
Recipient, 2010-11-25	72,235	57,469 / 14,766

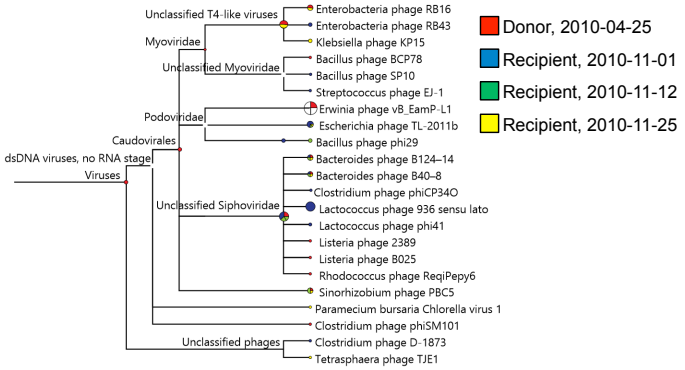
ORFs were predicted by GLIMMER3 [1]. The ORFs were compared against the NRPROT protein database with BLASTP [2], with ~80% hits in each dataset.

5. Microbial Composition of Donor and Patient



The highest-ranked BLASTP hits of each ORF in all of the four samples were subjected to taxonomical assignment with MEGAN [3]. Pie charts represent the distribution of deduced proteins assigned to bacterial phyla. Predominance of Firmicutes species is a characteristic of healthy intestinal microbiomes.

6. Detection of Viruses



In contrast to 16s rRNA-based metagenomics, our approach was capable of identifying viruses. We found about 10 viruses in each of the four stool samples and a total of 22 viruses, mostly phages.

7. Conclusions

- FT successfully cured recurrent CDAD with no side effects for three years
- Metagenomics by deep sequencing revealed a stable and diverse healthy intestinal microbiome in the patient
- no evidence for presence of *C. difficile* in patient and donor
- **Metagenomics by deep sequencing is a powerful tool to follow the result of FT**
- **More applications of the procedure may increase acceptance by authorities and people involved**

8. References

[1] Salzberg et al., 1998, NAR 26: 544.
[2] Altschul et al., 1990, JMB215: 403.
[3] Huson et al., 2007, Genome Res 17:377.

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