ISOLATION AND CHARACTERIZATION OF *Clostridioides difficile* FROM COMPOST SAMPLES

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Introduction

Clostridioides difficile is an important human pathogen, causing infections that are common in the hospitals. The rise of infection in the community have increased the interest to explore possible reservoirs and sources of *C. difficile* in domestic environments (1).

C. difficile spores are ubiquitous and are commonly found in different natural environments, animals, food and households (2,3).
We have previously shown that compost has the highest positivity rates in the domestic outdoor environment, 9 of 15 (60 %) compost samples from 5 domestic composters were positive for *C. difficile* (4).

The aim of this study was to explore the spatial and temporal distribution of *C. difficile* PCR ribotypes in compost piles sampled at two households from rural area.

Methodology

SAMPLING	Two composters (A and B) at two households from rural area in Slovenia were sampled from March 2018 to January 2019. Composter A was sampled 5 times and composter B was sampled 4 times. From each composter, five samples from different locations within compost pile were collected (Figure 1).
ISOLATION OF C. *difficile*	*C. difficile* was isolated without enrichment on CHROMID *C. difficile* plates (bioMérieux) using combination of temperature and alcohol chock and sonication (Figure 2). Each sample (25 g) was resupended in 60 ml of sterile distilled water and mixed well. Then 20 ml of resuspended sample was processed further (non-sonicated sample). To the remaining of the sample we first added 20 ml of distilled water and the sample was then subjected to sonication (30s in BactoSonic, Bandelin) after which 20 ml of the sample was processed further (sonicated sample). From here on all the steps are the same for sonicated and non-sonicated samples as described previously (4).
TYPING	All isolates were characterized by PCR ribotyping and toxinotyping.
WHOLE GENOME SEQUENCING AND SNV ANALYSIS	For whole genome sequencing, bacterial DNA was isolated with QIAamp DNA Mini Kit (Qiagen). Paired-end libraries were generated with Nextera XT Library preparation kit (Illumina) following manufacturer's protocol. Libraries were sequenced on MiSeq (Illumina) with 600-cycle MiSeq Reagent Kit v3. Whole genome single nucleotide variant analysis (wgSNV) was performed with the BioNumerics software v7.5 (Applied Maths). Dendrogram was constructed based on all retained SNVs using MEGA software.

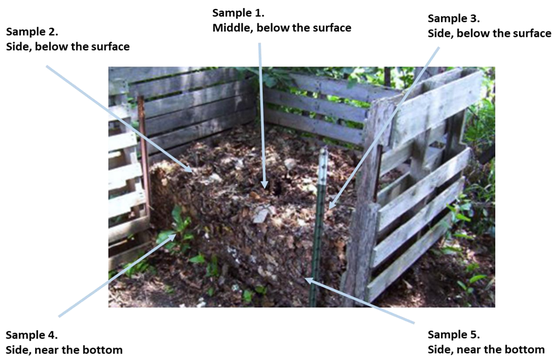


Figure 1. Schematic representation of sampling point within the composter.

25 g of compost
+ 60 ml water

WITHOUT SONICATION | SONICATION

Centrifugation

Temperature shock

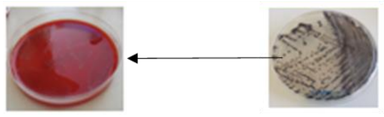
Centrifugation

Pellet on CHROMID *C. difficile*

Alcohol shock

Centrifugation

Pellet on CHROMID *C. difficile*



COH -> MALDI ID

Figure 2. Basic steps of sample processing.

Results

- Fourty-five compost samples were tested and ***C. difficile*** was **isolated** from 38 samples **(84.4%).**
- In total, **906 isolates** were obtained which were distributed into **35 PCR ribotypes.**
- The most common PCR ribotpyes were 018, 014 and SLO 057.
- From 3 to 16 different PCR ribotypes were found in a composter at different sampling times and up to nine different PCR ribotypes were found within a single compost sample.
- Only 5 PCR ribotypes were shared between the two composters; 001, 002, 011/049, 014/020 and 027 (Table 1).

Table 1. Presence of *C. difficile* PCR ribotypes within and between the two composters (A and B), humans, animals, environment and food in Slovenia.

PCR ribotype	Toxinotype	Composter A	Composter B	Humans	Animals	Environment	Food
001	0	+	+	+	+	+	+
002	0	+	+	+	+	+	+
003	0		+	+	+	+	
005	0	+		+	+	+	
010	0	+		+	+	+	
011/049	0	+	+	+	+	+	+
014/020	0	+	+	+	+	+	+
015	0		+	+	+	+	
018	0	+		+	+	+	
027	III (CDT+)	+	+	+		+	+
029	0	+		+	+	+	
045	V (CDT+)	+		+	+	+	
050	0	+		+	+	+	
070	0	+		+	+	+	+
078	V (CDT+)	+		+	+	+	
106	0	+		+		+	
128	III (CDT+)		+	+	+	+	
159	0	+		+			
764	V		+	+			
SLO 002	tox-	+		+	+	+	
SLO 028	tox-	+		+			+
SLO 038	0	+		+			
SLO 056	VIII	+		+		+	
SLO 057	tox-		+	+	+	+	+
SLO 069	0/v (CDT+)		+	+	+	+	
SLO 172	0/v (CDT+)		+	+		+	
SLO 205	tox-		+			+	+
SLO 218	tox-		+			+	
SLO 248	tox-	+		+			+
SLO 257	0	+		+			
SLO 259	tox-		+	+			
SLO 290	V (CDT+)	+					
SLO 291	tox-	+					
SLO 292	0		+				
SLO 293	0	+					

- In some instances the **same PCR ribotype** could be **found in multiple samples** in **the composter**, one of them is **001**.
- To check if these are **clonal isolates** we selected 4 isolates from composter A (from 4 samples) and one isolate from composter B for whole genome sequence analysis. The four isolates from composter A differed from 0 to 6 SNVs and were genetically unrelated to isolates from composter B.

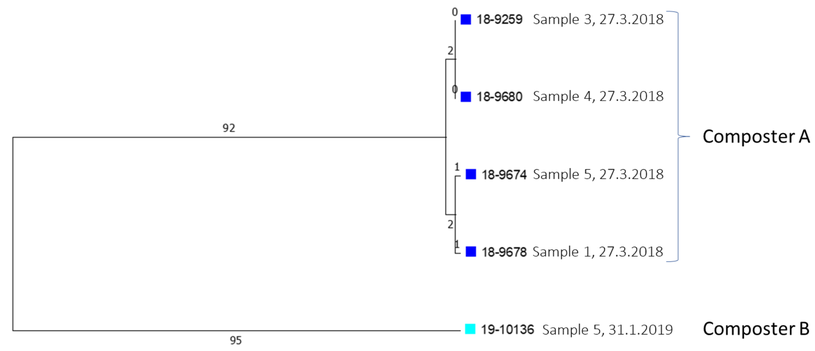


Figure 3. Single nucleotide variant analysis of five *C. difficile* isolates from two composters. Neighbor Joining tree is **scaled in SNVs.** Sample number corresponds to sample number in figure 1.

References

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Conclusion

Our result strongly suggest that *C. difficile* is commonly present in the domestic composters and adds to existing knowledge of potential sources of *C. difficile* in domestic environment.