

Appendix A

Canada DNA Samples Analysis Report

Note: In this report, only J1 is the sample corresponding to this thesis

1. Materials and method

1.1 Material

DNA samples from Canada were sequenced and analysed. Since tube of 31 was broken during delivery, sample 32 was sequenced as a substitute. The four DNA samples were renamed for the convenience of data analysis (table 1).

Table 1 Sample rename information

Sample name	Rename	Source
11	C11	Wastewater sludge
21	C21	Wastewater sludge
32	C32	Wastewater sludge
J1	CJ1	Algae-bacteria mixtures

1.2 Method

The four samples (11, 21, 32, J1) were sequenced by 16SV4 for bacteria community composition analysis.

2. Analysis of sequencing results on phylum level

This analysis revealed and compared the bacteria community composition of different samples in phylum level. Proteobacteria occupied large proportion in bacteria community, which was not clear for display results. Therefore, Proteobacteria was displayed on class level as showing in below table.

Table 2 Class display of Proteobacteria

Phylum	Proteobacteria
Class	Proteobacteria;Other

Alphaproteobacteria
 Betaproteobacteria
 Deltaproteobacteria
 Epsilonproteobacteria
 Gammaproteobacteria
 Proteobacteria;c__TA18

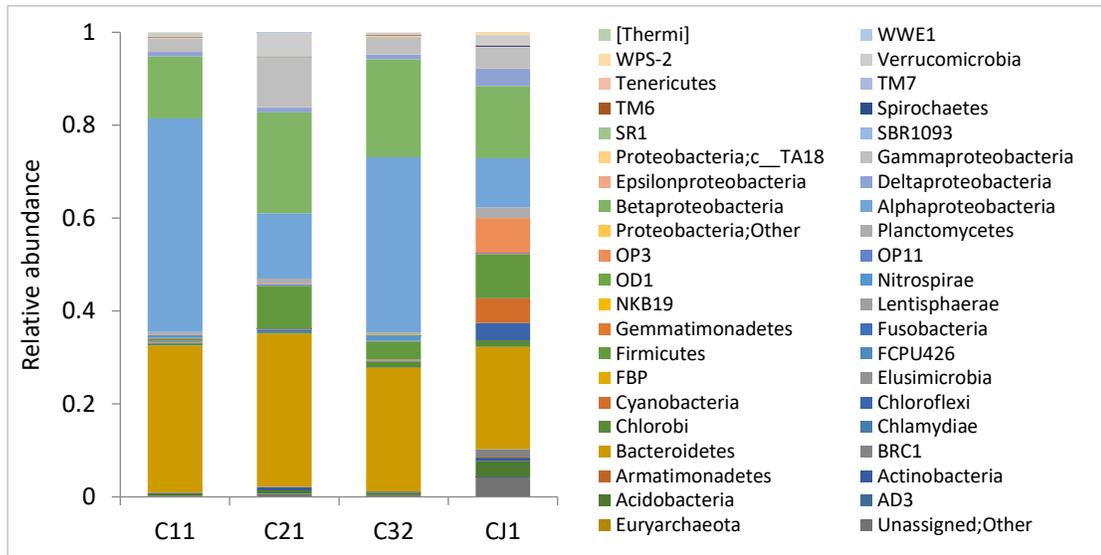


Figure 1 Compare of C11, C21, C32 and CJ1 of bacterial community composition in Phylum and Proteobacteria

Table 3 Dominant bacteria (relative abundance >0.02) in phylum level

Dominant bacteria	Relative abundance			
	C11	C21	C32	CJ1
Alphaproteobacteria	0.459527	0.140697	0.377753	0.105431
Bacteroidetes	0.316144	0.329201	0.266195	0.220665
Betaproteobacteria	0.13386	0.217112	0.210916	0.15513
Gammaproteobacteria	2.98E-02	0.107385	0.037594	0.045774
Firmicutes	--	0.091052	0.038839	0.094135
Verrucomicrobia	--	0.049483	--	--
OP3	--	--	--	0.075339
Cyanobacteria	--	--	--	0.053296
Unassigned;Other	--	--	--	0.043536
Deltaproteobacteria	--	--	--	0.037264
Chloroflexi	--	--	--	0.036825
Acidobacteria	--	--	--	0.03393
Planctomycetes	--	--	--	0.023227

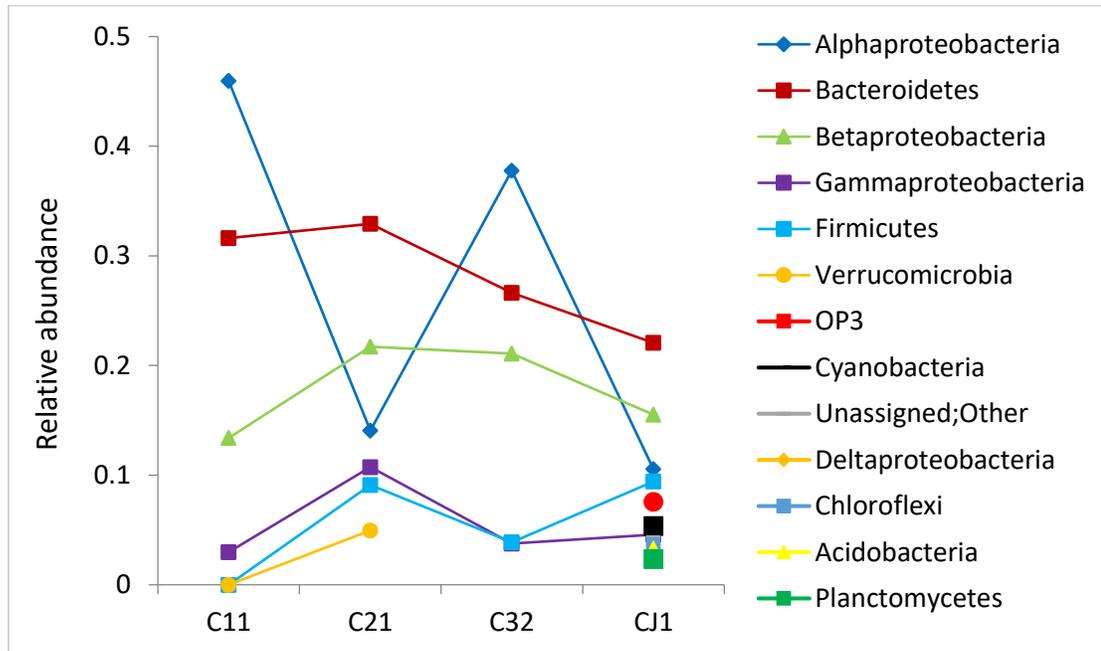


Figure 2 Relative abundance of dominant bacteria in phylum level

The compare of four samples in bacteria abundance was showing in figure 1. The dominant bacteria in phylum level (relative abundance >0.02) are showing as figure 2 and table 3.

- It can be seen that the bacteria community composition of CJ1 was much more abundant than the other three samples.
- Alphaproteobacteria, Bacteroidetes, Betaproteobacteria, Gmmaproteobacteria and Firmicutes were the five common phyla among the four samples.
- Verrucomicrobia was the dominant phylum only in C11 and C21.
- OP3, Cyanobacteria, Unassigned;Other, Deltaproteobacteria, Chloroflexi, Acidobacteria and Planctomycetes were the seven specific dominant phyla (relative abundance >0.02) in CJ1.
- The abundance fluctuation of dominant bacteria was indicated in figure 2.

3. Analysis of sequencing results on genus level

Since not all the data can be analysed to genus, the minimum analysed level was displayed in the following figures. This analysis revealed and compared the bacteria community

composition of different samples in the minimum analysed level. Here, c_, o_, f_ and g_ refer to class, order, family and genus respectively.

Since the data contained more than 500 items, the top 31 bacteria were considered as dominant ones (relative abundance of C11, C21, C32 or CJ1 >0.02) and analysed below. In figure 3, the sum of proportion of top 31 bacteria was set as 1.

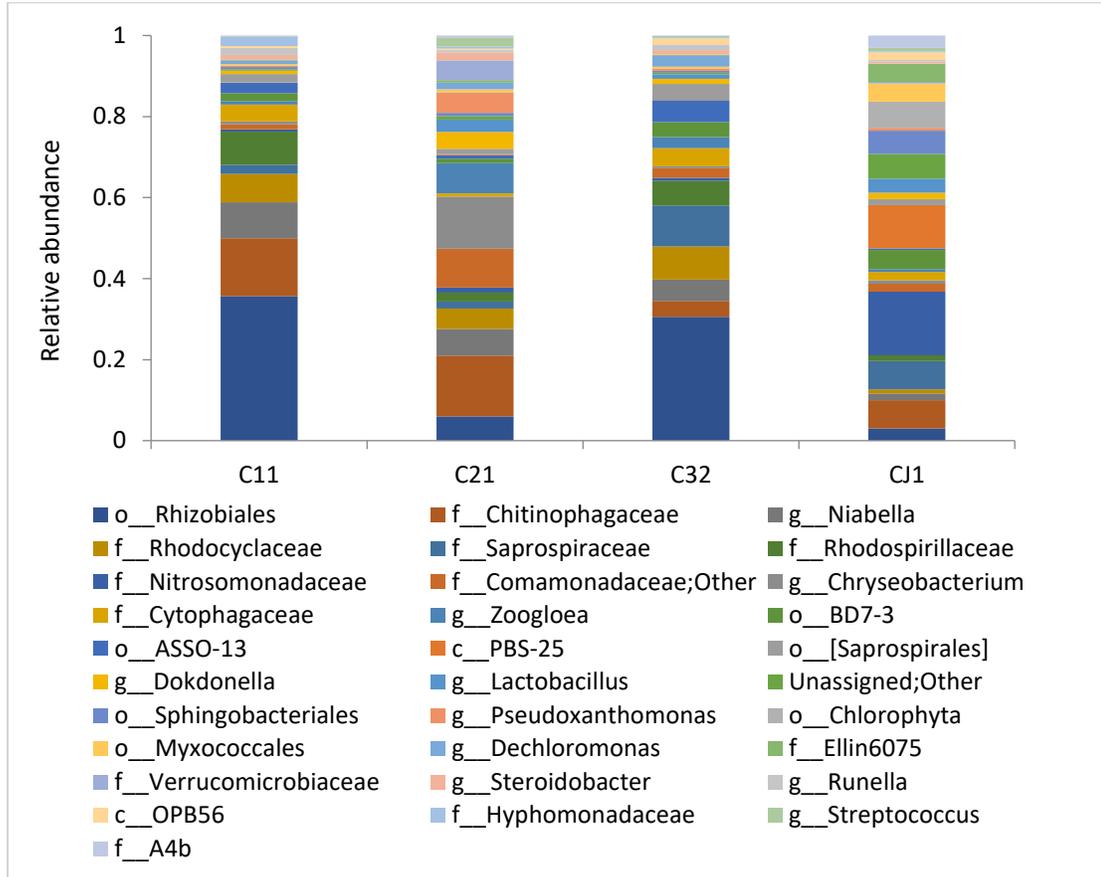


Figure 3 Compare of C11, C21, C32 and CJ1 of bacterial community composition in the minimum analysed level

Table 4 Dominant bacteria (relative abundance >0.02) in the minimum analysed level

Dominant bacteria	Relative abundance			
	C11	C21	C32	CJ1
o__Rhizobiales	0.317918	0.045829	0.261821	0.021472
f__Chitinophagaceae	0.127953	0.116107	0.033578	0.049173
g__Niabella	0.078946	0.050715	0.044834	--
f__Rhodospirillaceae	0.072455	--	0.05296	--
f__Rhodocyclaceae	0.062775	0.038561	0.070287	--
f__Cytophagaceae	0.036969	--	0.038179	--

o__ASSO-13	0.023156	--	--	--
f__Hyphomonadaceae	0.021539	--	--	--
f__Saprospiraceae	0.020259	--	0.086218	0.049743
g__Chryseobacterium	--	0.099087	--	--
f__Comamonadaceae;Other	--	0.073569	0.020117	--
g__Zoogloea	--	0.057236	0.023737	--
g__Pseudoxanthomonas	--	0.03844	--	--
f__Verrucomicrobiaceae	--	0.037975	--	--
g__Dokdonella	--	0.031919	--	--
g__Lactobacillus	--	0.023863	--	0.024345
o__ASSO-13	--	--	0.046512	--
o__[Saprospirales]	--	--	0.033428	--
o__BD7-3	--	--	0.031712	0.033798
g__Dechloromonas	--	--	0.022964	--
f__Nitrosomonadaceae	--	--	--	0.111791
c__PBS-25	--	--	--	0.075339
o__Chlorophyta	--	--	--	0.045774
Unassigned;Other	--	--	--	0.043536
o__Sphingobacteriales	--	--	--	0.040663
o__Myxococcales	--	--	--	0.03268
f__Ellin6075	--	--	--	0.031671
f__A4b	--	--	--	0.021713
Amount	9	11	13	13

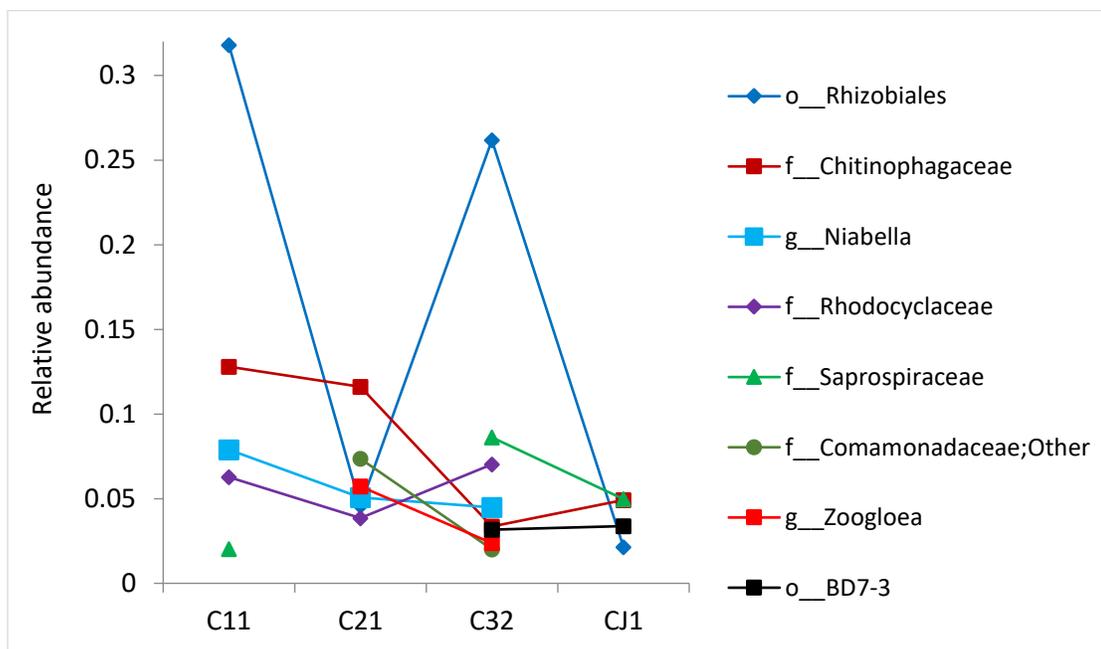


Figure 4 Relative abundance of dominant bacteria in the minimum analysed level

In this section, bacterial community composition of C11, C21, C32 and CJ1 was discussed in the minimum analysed level.

- a) Compare of the abundance of the top 31 bacteria (relative abundance of C11, C21, C32 or CJ1 >0.02) was showing in figure 3.
- b) The dominant bacteria (relative abundance of C11, C21, C32 and CJ1 >0.02) was showing in table 4 and figure 4.
- c) The abundance fluctuation of dominant bacteria was indicated in figure 4.

4. PC analysis

PC analysis could reveal the similarity of different bacteria community in three dimensions. Since the explanation degree of PC1 showed the largest value (43.59%), dimension of PC1 can be based for analysis.

- a) It can be seen from figure 5 that C21, C32 and CJ1 indicated more similarity in PC1 dimension.
- b) C11 showed less similarity compared with the other samples.

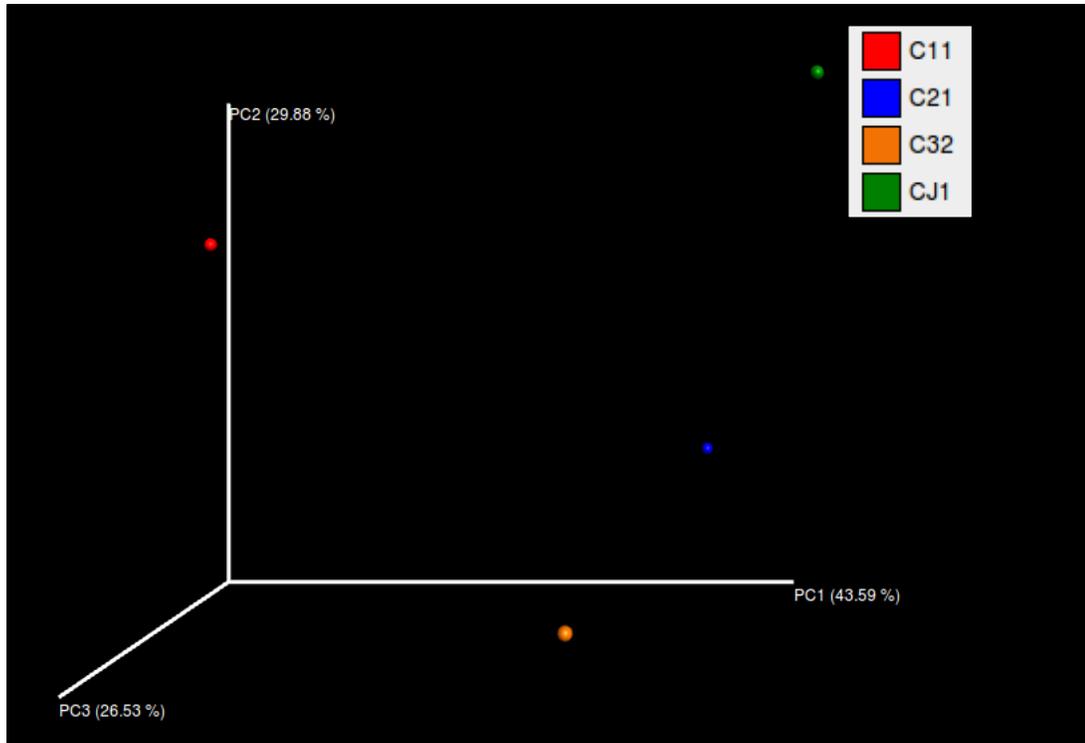


Figure 5 PC analysis of C11, C21, C32 and CJ1

5. LEfSe analysis

LEfSe analysis was carried out to reveal the differences of bacteria community composition among C11, C21, C32 and CJ1. Cladogram and bar chart were plot to indicate the results.

Cladogram (figure 6)

- a) The points with same colour revealed the similarity of bacteria composition in C11, C21, C32 and CJ1.
- b) The points with different colour indicated the differences of bacteria composition in the four samples.
- c) The specific bacterium that related to different colour/code can be found from the list in the right hand.

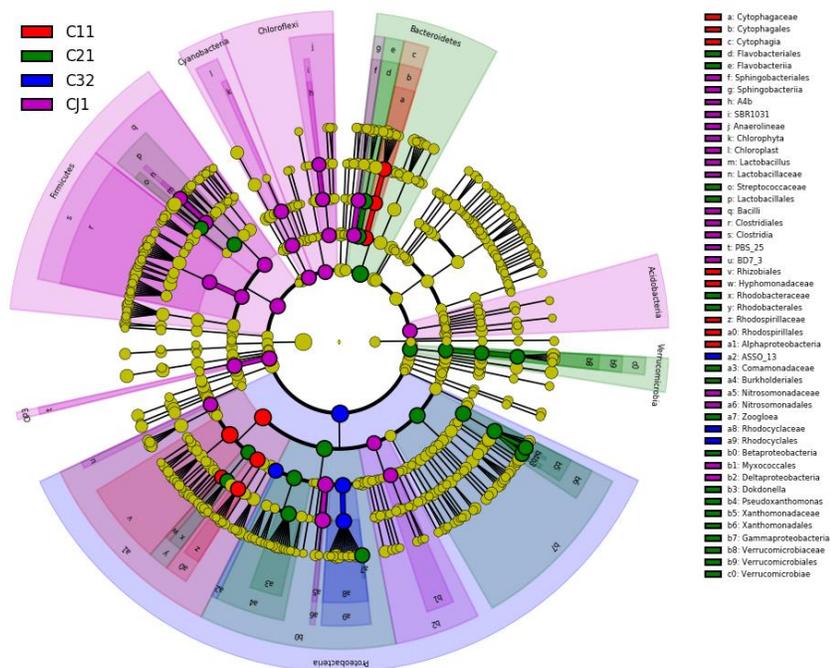


Figure 6 The Cladogram of C11, C21, C32 and CJ1 by LefSe analysis

Bar chart (figure 7)

- It revealed the differences of bacteria community composition among C11, C21, C32 and CJ1.
- LDA score indicated the degree of different, which did not show the relationship with bacteria abundance.
- The different bacterium listed in figure 7 included all levels from phylum to genus.
- The bacteria community composition of C11, C21, C32 and CJ1 showed differences as showing in figure 7.

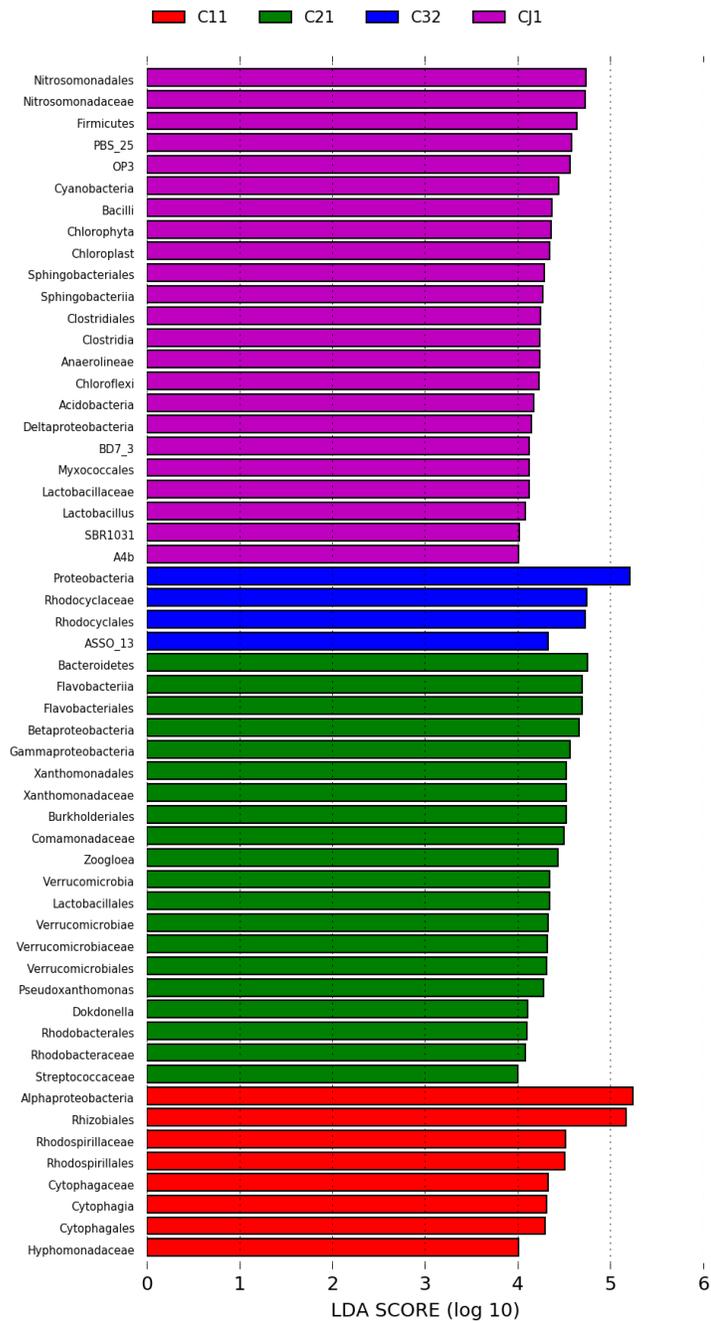


Figure 7 The differences of bacteria community composition among C11, C21, C32 and CJ1 by LEfSe analysis