(45) Comparison of Bacterial Communities in Treated Water and in Activated Sludge

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The present study focused to grasp bacterial communities in activated sludge and treated water. Activated sludge and treated water (effluent) samples were obtained from two laboratory reactors and from a full scale wastewater treatment plant. Bacterial community structures in the samples were profiled by polymerase chain reaction (PCR) targeted at partial 16S rRNA gene followed by terminal-restriction fragment length polymorphism (T-RFLP). While many of the peaks were shared in T-RFLP profiles from activated slduge and treated water samples, significant differences were also found. Some of the peaks were found more in treated water, suggesting that their corresponding bacteria were dominant in treated water. Further studies are needed to understand differences of bacterial communities in activated sludge and treated water, especially when we consider the increasing needs for the reuse of treated water.

Key Words : *Bacterial population, activated sludge, treated water, 16S rRNA gene, T-RFLP*

1. INTRODUCTION

Activated sludge processes are widely employed for wastewater treatment. Activated sludge processes use diverse group of microorganisms that form aggregates named flocs. Treated water and activated sludge are typically separated either by filtration or by gravimetric settling. Except for membrane activated sludge processes, water treated by activated sludge is separated only by gravimetric settling, and thus small amount of microorganisms remain in treated water. These microorganisms in treated water affect the quality of treated water in three points of view. First is from the view to control health-related microbial indicator: they will increase heterotrophic bacterial counts in treated water[1), 2)]. Secondly, microorganisms in treated water might affect ecosystems in receiving water bodies. Thirdly, these microorganisms in treated water can interfere with advanced treatment processes for its reuse[3)]. Especially, considering the latter two points, knowledge not only on health-related microorganisms but also on whole microbial communities in treated water is essential.

However, knowledge on the whole microbial population and their behavior in treated water is rather scarce. There are engineers who simply presume that bacterial population in treated water is quite similar to that in activated sludge. However, Müller et al. reported that smaller flocs in activated sludge treating paper mill wastewater had different community structures compared to larger flocs[4)]. In addition, Wilén et al. reported that some groups of bacteria in *Gammaproteobacteria* were more loosely bound to flocs[5)]. These studies suggest the possibility that microbial communities in activated sludge and in treated water are somewhat different.

Apparently, in spite of the potential needs, understanding on whole microbial population in treated water is missing. In the present study, the authors attempted to study the differences of bacterial population in activated sludge and in treated water. Samples were obtained from laboratory scale activated sludge reactors and a full scale wastewater treatment plant (WWTP). We selected these three systems so that we can show evidences for plural cases. Samples were obtained in time series to see their dynamics. Bacterial populations were analyzed by the polymerase chain reaction (PCR) targeted at a partial sequence of 16S rRNA gene followed by T-RFLP method.

2. MATERIAL AND METHODS

(1) Nature of the samples

Activated sludge and treated water samples were processed for microbial analyses. Both activated

sludge and treated water samples were collected from two laboratory reactors and a full scale WWTP in Kanto area, Japan. Two laboratory reactors, Reactor 1 and Reactor 2, were operated as sequencing batch reactors (SBR) with a working volume of 10 liters with sequencing anaerobic and aerobic conditions. These reactors were operated with 6 cycles per day including the basic phases of influent supply followed by an anaerobic, an aerobic, settling, and treated water discharge. The reactors were installed in an air-conditioned room controlled at around 20-24°C. For laboratory reactors, seed sludge was obtained from actual WWTPs and the influent to the reactors was synthetic wastewater. The synthetic wastewater[6), 7)] for Reactor 1 (and Reactor 2 in parentheses) contained 90mg/L (368mg/L) of $CH_3COONa{\cdot}3H_2O$, 43mg/L (130mg/L) of CH_3CH_2COONa, 80 mg/L (14mg/L) of peptone, 16 mg/L (17mg/L) of yeast extract, 29mg/L (71mg/L) of K_2HPO_4, 11 mg/L (12mg/L) of $CaCl_2{\cdot}2H_2O$, 88mg/L (75mg/L) of $MgSO_4{\cdot}7H_2O$, and 34mg/L (30mg/L) of KCl. In addition, the feed to Reactor 2 contained 75mg/L of NH_4Cl, 123mg/L of $NaHCO_3$, 0.4 mg/L of allylthiourea, and trace elements with 0.375mg/L $FeCl_3{\cdot}6H_2O$, 0.0375mg/L H_3BO_3, 0.0075mg/L $CuSO_4{\cdot}5H_2O$, 0.045mg/L KI, 0.03mg/L $MnCl_2{\cdot}4H_2O$, 0.015mg/L $Na_2MoO_4{\cdot}2H_2O$, 0.03mg/L $ZnSO_4{\cdot}7H_2O$, 0.0375mg/L $CoCl_2{\cdot}6H_2O$, and 2.5mg/L EDTA. Sludge retention times were not so exactly controlled, but were around 10 days, and hydraulic retention times were 8 hours. Mixed liquor suspended solids (MLSS) concentrations were in between 750mg/L and 1,500mg/L. Activated sludge samples were collected at the end of aerobic phase, while treated water samples were collected form the discharged treated water. For each reactor, activated sludge sample and treated water samples were obtained once a week for four weeks. Reactor 1 had been operated for about half year prior to the experimental period. Operation of Reactor 2 was started only one day before the first sampling.

The full-scale WWTP was operated also in the sequencing anaerobic and aerobic conditions with urban wastewater as the influent. This WWTP is different from the one from which seed sludge for the laboratory reactors were obtained. Samples were obtained daily from Feb.15 (Mon.) through Feb.19 (Fri.), 2009. To reduce the load for sampling, the authors asked the plant operators to collect activated sludge mixtures at the end of the aeration tank. The supernatant of the activated sludge mixture was used as the "treated water" sample. MLSS concentrations were around 1,000mg/L.

(2) Microbial analyses

a) DNA Extraction

Bacterial DNA in activated sludge samples were extracted by the sonication-dilution method[8)]. Activated sludge mixed liquor, 1mL, was poured into a 50mL-size plastic tube, 29mL Milli-Q water was added to it, and sonicated with an Advanced-Digital Sonifier 250 (Branson, USA) equipped with a 1/2 inch horn at an amplitude value of 100% for 30s.

Treated water sample, 30mL was poured into a 50mL-size plastic tube without dilution, and sonicated with the same sonifier and the horn at an amplitude value of 100% for 30s.

The concentration of templates DNA was around 10,000pg/μL and 1,000pg/μL in activated sludge and treated water samples from laboratory reactors respectively after quantified using Pico-Green dsDNA Quantification kit (Invitrogen) according to the manufacturer's instructions. Template DNA concentration of activated sludge and treated water of the full scale WWTP was around 10,000 pg/μL and 100 pg/μL respectively. The sonicated samples were used as the template for PCR reaction without purification.

b) PCR

Partial 16S rRNA gene was amplified with the universal primer set of 27f (5'-AGAGTTTGATCM-TGGCTCAG) labeled with FAM (phosphor-amidite fluorochrome 5-carboxy-fluorescein) on the 5' end and 519r (5'- GWATTACCGCGG-CKGCTG) primer pairs[9)]. The 25μL PCR mixture contained 1× PCR buffer, 0.2mM dNTP, 0.2μM forward and reverse primers, 0.025U/μL Ampli Tag Gold (Applied Biosystems, Inc., USA), and 2.5μL of DNA template. PCR reaction was performed with a Thermal Cycler Dice (Takara, Japan) and an thermal program of 95°C for 600s followed by 30 cycles of (94°C for 30s, 55.3°C for 30s and 72°C for 30s) followed by 72°C, 600s.

c) T-RFLP

The PCR products were purified using QIAQuick PCR Purification kits according to the manufacture's instruction. Restriction enzyme digestion was performed with *RsaI* restriction enzyme (Takara, Japan) at a 10μL size including 5μL of purified PCR product (around 65 ng of DNA) 1μL of 10×restriction buffer, 1.6 U of restriction enzyme and sterilized Milli-Q water at 37°C for 4 hours followed by heating at 65°C for 15 minutes. Digested samples were pretreated with DNA marker (GeneScan 500 ROX Size Standard, Applied Biosystems) and HiDi Formamide (Applied Biosystems). Finally, samples were analyzed using

ABI310 (Applied Biosystems) to obtain the electropherograms of each sample[10),11)].

(3) PCA analysis

Principal component analysis (PCA) was performed for the data of full scale WWTP to visualize the differences of microbial populations among the samples and trend of their changes. The MarkerView software (AB Sciex, USA) was used to perform the PCA analysis.

3. RESULTS AND DISCUSSION

Bacterial population structures in the analyzed samples are shown in Figs. 1 and 2 for weekly sampled laboratory reactors, and 3 for daily sampled from full scale WWTP. In each Figure, (a) shows bacterial population structures in activated sludge, and (b) shows those in treated water. In theory, each bacterial species are corresponded with one single peak[9)], and thus, the differences in the peak pattern are translated as the differences in bacterial population structure. In all the profiles, peaks were named as relevant base pairs of each peak.

As can be seen in Fig. 1(a) and Fig. 2(a), gradual changes of bacterial communities in activated sludge were observed. Change of the peak intensities for peak 307 (a peak with a size of 307 bases) in Fig. 1(a) and peaks 94, 163, 303 in Fig. 2(a) were clearly seen, indicating the change of abundances of bacterial species corresponding to these peaks. However, when compared the patterns of the peaks such named as, 91, 163, and 414 in Fig. 1(a) and 481 in Fig. 2(a) dynamics of those bacterial species were almost independent.

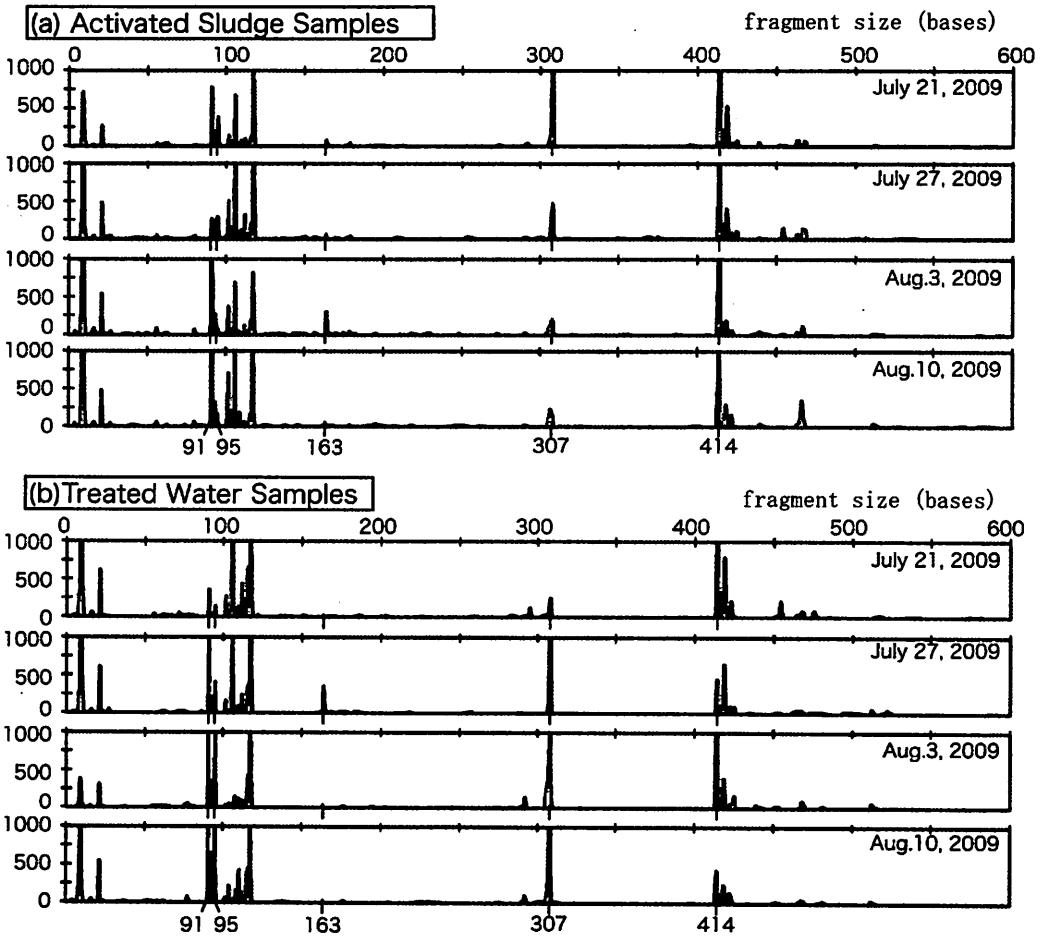


Fig.1 T-RFLP profiles of the bacterial communities in activated sludge and treated water from laboratory Reactor 1.

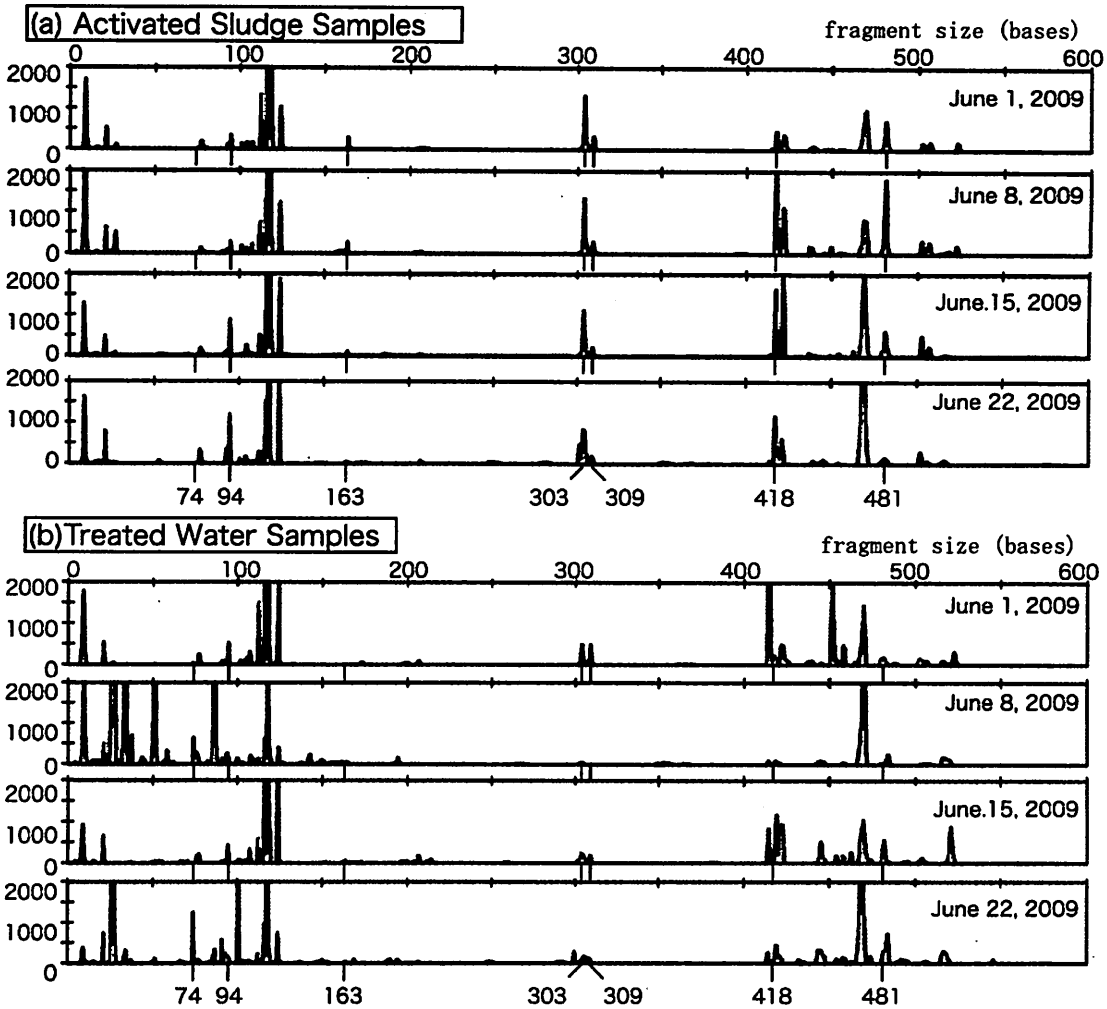


Fig.2 T-RFLP profiles of the bacterial communities in activated sludge and treated water from laboratory Reactor 2.

As can be seen in Fig. 1(b) and Fig. 2(b), many of the peaks found in activated sludge samples were also found in treated water samples, supporting the presumption that many of bacteria in treated water are from activated sludge. Yet, not small differences were also found. Peaks sized at 91 and 307 bases in Fig. 1 and those sized at 74, 418 and 481 bases in Fig. 2 were intensified in treated water suggesting their corresponding bacterial communities were more abundant in treated water samples. These bacteria may be pin-floc-related or free living bacteria. In Fig. 1, corresponding bacterial community for peak 307 base was more abundant in the treated water sampled on August 3rd and August 10th 2009 compared with those in activated sludge in these two days. In Fig. 2, the corresponding bacterial communities for peaks 303 and 309 bases were found less in the treated water, which imply they are related to more stable flocs.

As shown in Fig. 3, bacterial populations in activated sludge and in treated water were different also in the full scale WWTP samples. Even though, most of the peaks were commonly found in all T-RFLP profiles, peak 118 bases was more intense in treated water than in activated sludge and peak 124 bases was more intense in activated sludge. In addition, the peak patterns in the range with fragment size less than 50 bases were consistently different between activated sludge and treated water samples.

Because samples from the full scale WWTP were obtained for successive five days, while those from laboratory reactors were obtained weekly for four weeks, it was expected that microbial population structures were more consistent during the monitoring period, and would be useful to

examine if there are any clear trend in the microbial population structure. In order to visualize trends in microbial population shift in samples from the full scale WWTP, the T-RFLP data were further analyzed by PCA analysis. As there was a fear that peaks with shorter fragment sizes were affected by desalination step in sample preparation, we used data for fragment peaks bigger than 50 bases. As shown in Fig. 4(a), plots for sludge samples were located on in the region with negative PC1 score, while those for treated water samples in the region with positive PC1 score. And plots for sludge samples had a consistent trend to move downward to lower PC2 scores during the monitoring period. Plots for treated water samples also had similar trend, though PC2 score increased between Feb. 16 and 17. PC1 score is thought to characterize the difference of bacterial community structures in sludge and treated water samples. As can be seen in Fig. 4(b), peak 118 and 124 in T-RFLP profiles contributed significantly to the PC1 scores as was expected. PC2 score is thought to be corresponding to the temporal change of microbial community in activated sludge during the monitored period. To PC2 score, the contributions of peaks 243, 304, 309, and 485 were higher.

4. CONCLUSION

The outcomes clearly suggested that bacterial community structure in treated water has significant differences from that in activated sludge. Some of

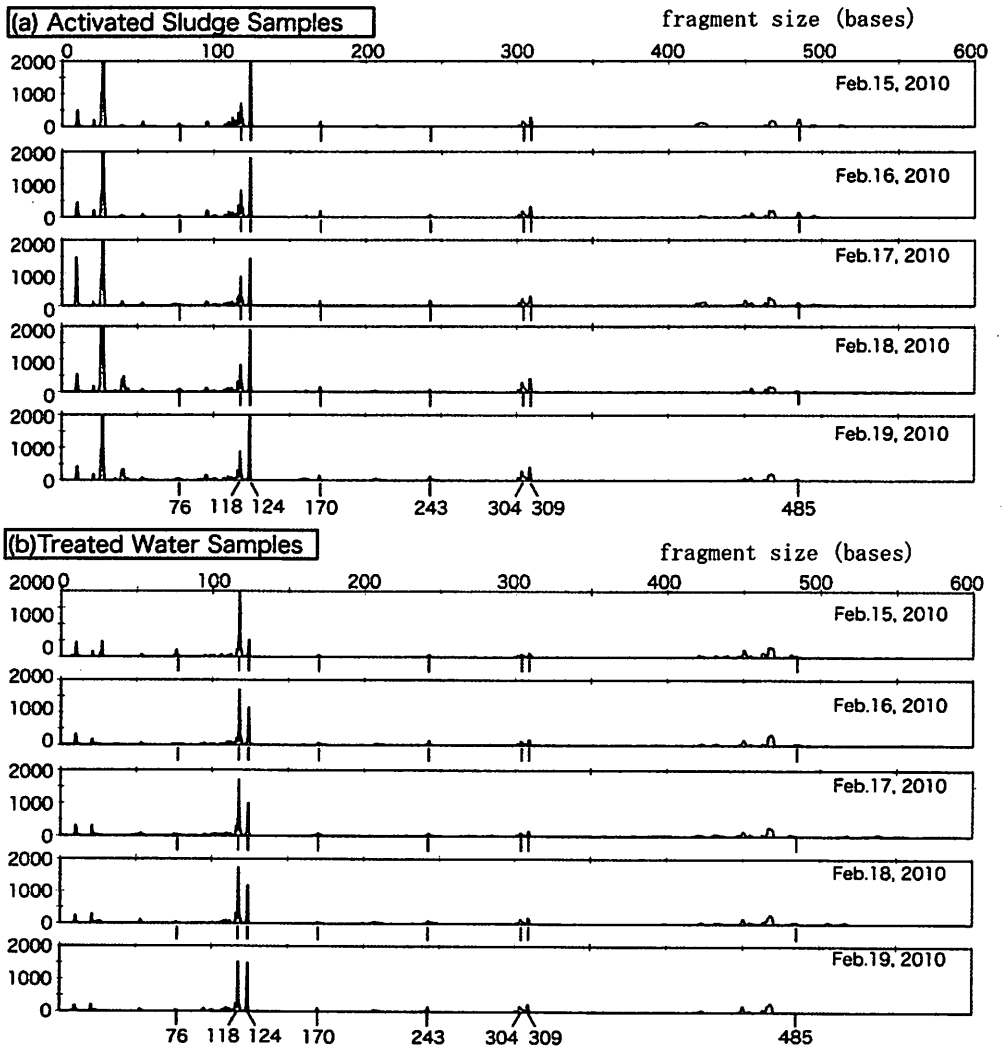
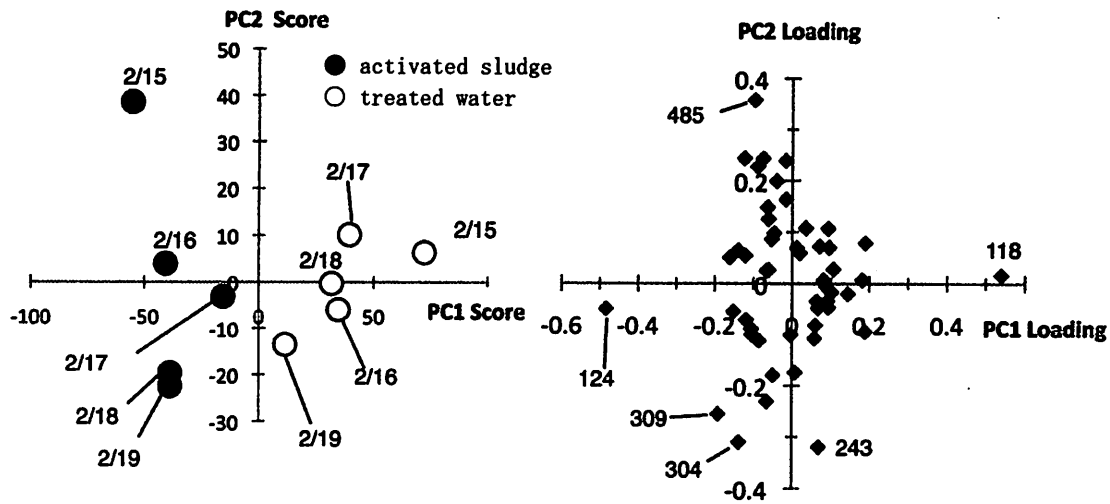


Fig.3 T-RFLP profiles of the bacterial communities in activated sludge and treated water from the full scale wastewater treatment plant.



(a) Score plot for the samples.

(b) Loading plot for the T-RFLP peaks.

Fig. 4 PCA analysis of the T-RFLP profiles for the samples from the full scale WWTP. In loading plot, the sizes of peaks with smaller loading values are omitted.

peaks were found more intense in treated water, meaning bacterial species corresponding to these peaks are associated with pin flocs or freely living in the bulk liquid. Other peaks were found to be more intense in activated sludge, and bacteria corresponding to these peaks may be associated with more stable flocs. The PCA analysis showed that there was a similar trend in bacterial population changes in activated sludge and in treated water in the full scale WWTP.

More study would be needed to more clearly understand the behavior of bacterial communities in water treated by activated sludge processes, such as the impact of operational and environmental conditions. In addition, major bacterial species in treated water should be phylogenetically clarified.

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