Development of DNA chip for species identification in the activated sludges

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Abstract

The activated sludge process is the most essential process for treating domestic and industrial effluents within most wastewater treatment plants. Since the activated sludge process was developed, several problems have been observed, namely sludge foaming and bulking. Several factors are known to affect these, mainly, the uncontrolled overgrowth of filamentous bacteria. There are several treatment strategies to prevent these problems. However, these are not long-term solutions, because the main factors stimulating growth of filamentous bacteria are not known. For these reasons, it is essential and important to monitor the population of specific sludge bacteria to efficiently process activated sludge before bulking and foaming problems occur 1).

Norcardia sp, *Microthrix parvicella*, and several type strains are filamentous bacteria and well known to induce bulking and foaming. Among these, *Norcardia* (*Gordonia*) *amarae* is well characterized in terms of initiating bulking and foaming during the activated sludge process. To observe this species' population in the activated sludge, several technologies were developed (e.g. Fatty acid confirmation analysis, antibody assay, rRNA probe hybridization methods, and morphological feature analysis). In particular, the fatty acid confirmation analysis and antibody assay only focus on one species identification and population check. When considering the complex populations within activated sludge, a screening tool for complex populations that is easier than other methods is needed.

We integrated specific DNA probes on a 0.5 cm X 1 cm glass slide to develop species identification tools for use with activated sludge. First, we used three model bacteria: *Norcardia* (*Gordonia*) *amarae*, *Mycobacterium pergernium*, and *Zooglea ramigera*, which are well known to grow in the activated sludge. Using random shotgun cloning using fragmented genomic DNA,

we obtained more than 50 probes for each strain. A total of 150 probes were arranged on the glass slide to discriminate each strain from another. For the reference signals, all three genomic DNAs were primed with cy5-dCTP and each DNA sample was primed with cy3-dCTP. For primed fragment hybridization, we use common microarray hybridization methods.

Scanning results and statistic analyses show that this system can identify the presence of specific strains via their genomic DNA well. Furthermore, we assessed the use of mixed genomic samples (two or three species) using this system, and also found this system is capable of identifying a species within a mixed population.

This tool will be useful to monitor the specific bacteria and its abundance in an activated sludge process and offer information on how the activated sludge system should be operated to minimize problems.

References

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