Abstract

Cyanuric acid is a key intermediate in the biodegradation pathway of atrazine which has been detected in ground and surface waters because of its widespread use around the world. Few strains are known for their ability to degrade and mineralize atrazine. Some of these strains harbor the atzD and trzD genes, which encode for cyanuric acid amidohydrolase, essential for cyanuric acid mineralization into carbon dioxide and ammonia. For more than 20 years, research has revealed that urea is one of the intermediates in cyanuric acid degradation. However, recent studies suggest that urea is not produced during the metabolism of atrazine or cyanuric acid.

In this thesis, hundreds of isolates capable of degrading cyanuric acid were isolated and purified using direct plating method and a clearing zone assay. Using rep-PCR, these isolates were clustered into groups based on their DNA fingerprint patterns. Of these, 25 new strains were found to represent all of these isolates. The sequence analysis of the 16S rRNA gene indicated that the 25 isolates represented bacteria in seven genera: *Alcaligenes* sp., *Tetrathiobacter kashmirae*, *Klebsiella* sp., *Ochrobactrum anthropi*, *Agrobacterium tumefaciens*, *Gordonia* sp., and *Achromobacter* sp. Among these genera, *Alcaligenes* sp. and *Tetrathiobacter kashmirae* represented the majority of the strains, while *Tetrathiobacter* sp., *Ochrobactrum* sp., *Gordonia* sp., and *Achromobacter* sp. were previously unknown to have the ability to metabolize cyanuric acid.

Studies done using the polymerase chain reaction indicated that the newly isolated cyanuric acid-catabolizing bacteria contained only homolog of *trzD*, and *atzD* was not detected. Sequencing 88% of *trzD* genes indicated that they were 100% identical to *trzD* from *K.*

pneumoniae strain 99 and *Pseudomonas* sp. strain NRRL B-12227, and highly conserved among the isolated strains degrading cyanuric acid.

PCR analysis indicated that several other genes in the degradation pathways of both atrazine and cyanuric acid were notfound, except for *atzC*, encoding N-isopropylammelide isopropylamiohydrolase. The *atzC* was found in 30% of the newly isolated cyanuric acid degrading bacteria. Surprisingly, PCR results indicated the absence of *atzE*, *atzF*, *trzE* and *trzF* genes, which are essential for mineralizing biuret and allophanate in the lower part of cyanuric acid degradation pathway. However, strains were still able to degrade cyanuric acid. Therefore, these results suggest that either there exists a new set of genes that are encoding forbiuret and allophanate catabolism in the newly isolated strains, or that the genes present have diverged from the ones we commonly know about.1st

Studies done using a closed system and ^{14}C-labeled-cyanuric acid as a source of nitrogen indicated that cyanuric acid was completely mineralized. Studies done using HPLC to monitor the metabolites of cyanuric acid degradation indicated the absence of urea as a byproduct, while biuret and allophanate were the main metabolites of cyanuric acid degradation detected in three of the newly isolated strains. Taken together, results of my studies done using different approaches and tools confirmed that the newly isolated strains completely mineralized cyanuric acid, and suggest that other unknown genes may be involved in the mineralization of cyanuric acid degradation to carbon dioxide and ammonia.