Proc. KMB Annual Meeting
June 24-26, 2003, Muju, Korea

N-Acyl Homoserine Lactone-Degrading Enzymes for Anti-Quorum Sensing in *Bacillus thuringiensis* and *Arthrobacter* sp.

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Quorum-sensing is a signaling mechanism that controls diverse biological functions, including virulence via *N*-acyl-homoserine lactone (AHL) signal molecules in gram-negative bacteria. Since AHL-mediated signaling mechanisms are widespread and highly conserved in many pathogenic bacteria, they can be attractive targets for novel anti-infective therapies. Therefore, the inactivation of AHL itself is the most obvious strategy among intervention strategies for disrupting quorum-sensing in bacteria.

Recently, the *aiiA* gene encoding an enzyme catalyzing the degradation of AHL has been cloned from *Bacillus* sp. 240B1. From the *B. thuringiensis* subsp. *morrisoni* genome, an *aiiA* homologue gene in the genome sequence was found. These results led to consideration of the possibility of the widespread existence of the gene in *B. thuringiensis*. *aiiA* homologue genes were found in 16 subspecies of *B. thuringiensis,* and their sequences were determined. Comparison of the *Bacillus* sp. 240B1 *aiiA* gene with the *B. thuringiensis aiiA* homologue genes showed high homologies of 89–95% and 90–96% in the nucleotide sequence and deduced amino acid sequence, respectively. Among the subspecies of *B. thuringiensis* having an *aiiA* gene, *aizawai, galleriae, kurstaki, kyushuensis, ostriniae,* and *subtoxicus* were shown to degrade AHL. It was observed that recombinant *E. coli* producing AiiA proteins also had AHL-degrading activity, and could also attenuate the plant-pathogenicity of *Erwinia carotovora*. In conclusion, genes for AHL-degrading enzyme in the *B. thuringiensis* are widely distributed in many subspecies of *B. thuringiensis*. Until now, *B. thuringiensis* has been developed and is used as a biological control agent against only insect pests in the agriculture and forestry industries. Our results suggest that insecticidal *B. thuringiensis* strains might have potential to be developed as a biological control agent against plant-pathogenic bacteria such as *Erwinia carotovora.*

Also, as a source of AHL-degrading enzymes, microorganisms are increasingly being investigated and we have been interested in isolating novel AHL-degrading bacteria for the purpose of disrupting and manipulating quorum-sensing signaling in agricultural pathogenic bacteria. Novel AHL-degrading bacteria were screened for AHL degradation by their ability to utilize *N*-3-oxohexanoyl-L-homoserine lactone (OHHL) as the sole carbon source. Among four isolates, strain IBN110, identified as *Arthrobacter* sp., was found to grow rapidly on OHHL, and exhibit degrading activity towards various AHLs with different lengths and acyl side chain substitutions. Coculture with the *Arthrobacter* sp. IBN110 and *Erwinia carotovora* significantly reduced both the AHL amount and pectate lyase activity in coculture medium, suggesting the possibility of applying *Arthrobacter* sp. IBN110 to the control of AHL-producing pathogenic bacteria. The *ahlD* gene from *Arthrobacter* sp. IBN110 encoding the enzyme catalyzing AHL degradation was cloned, and found to encode a protein of 273 amino acids. A mass spectrometry analysis showed that AhlD likely hydrolyzes the lactone ring of *N*-3-hexanoyl-L-homoserine lactone, indicating that AhlD is an

AHL-lactonase. A comparison of AhlD with other known AHL-degrading enzymes, *Bacillus* sp. 240B1 AiiA, a *Bacillus thuringiensis* subsp. *kyushuensis* AiiA homologue, and *Agrobacterium tumefaciens* AttM, revealed 25, 26, and 21% overall identities in the deduced amino acid sequences, respectively. Although these identities were relatively low, the HXDH≈H≈D motif was conserved in all the AHL-lactonases, suggesting that this motif is essential for AHL-lactonase activity. From a genome database search based on the conserved motif, putative AhlD-like lactonase genes were found in several other bacteria, and AHL-degrading activities observed in *Klebsiella pneumoniae* and *Bacillus stearothermophilus*. Furthermore, it was verified that *ahlK*, an *ahlD* homologue gene, encodes AHL-degrading enzyme in *Klebsiella pneumoniae*. Accordingly, the current results suggest the possibility that AhlD-like AHL-lactonases could exist in many other microorganisms.

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