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## **Substrate Specificity of Adenylation Domains Included in NRPS Modules of Cephacillin Biosynthetic Gene Cluster**

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Cephacillins, the new class of cephem antibiotics produced by Gram-negative bacilli, have oligopeptide at C-3 position of cephem ring differently from other cephem compounds. It can be assumed that the oligopeptide side chain of cephacillins is biosynthesized by large multifunctional enzymes called nonribosomal peptide synthetase (NRPS). The complete gene cluster involved in the biosynthesis of cephacillins in *L. lactamgenus* was cloned. Particularly in the 24-kb upstream region of *pcbAB* gene for ACV synthetase, the genes for 4 NRPS modules, 1 polyketide synthase (PKS) module and 2 ATP-binding cassette (ABC) transporters were deduced by comparison of sequence homology. In *cpbI* gene, 3 NRPS modules and 1 PKS module were identified (15,147 bp), encoding a hybrid NRPS/PKS protein with deduced size of 570 kDa. A putative *cpbK* is 3,168 bp in size, encoding another NRPS with deduced size of 115 kDa. In order to predict the substrate (amino acid) specificity of each NRPS module, the adenylation domain (AD) genes were cloned into pET expression system and expressed in corresponding *E. coli* host strain. The over-expressed recombinant ADs were purified through nickel affinity column chromatography, and subjected to ATP-PPi exchange assay for the examination of the enzymatic activities and substrate specificities. The results showed that AD1 have high substrate specificity for L-Arg and AD2, 3 and 4 for L-Ala, which is much consistent with the order of amino acids in oligopeptide chain of cephacillin. However, 4 ADs did not show strict substrate specificity for certain amino acid. It may be attributed to *in vitro* test for AD only instead of natural NRPS protein, but it cannot be excluded that the flexible substrate specificities of ADs may give diverse structure of cephacillin.