[S5-4] [10/11/2003(Sat) 11:50-12:30/ Grand Ballroom 102]

Biosynthetic Gene Cluster of Cephabacin for the Combinatorial Biosynthesis of β-Lactam Antibiotics

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β-Lactams are historically and clinically representative antibiotics used for therapeutic purposes. In early days, penicillin (penam antibiotic) and cephalosporin (cephem antibiotic) were found in culture broth of two different filamentous fungi, *Penicillium chrysogenum* and *Acremonium chrysogenum*. Since 1970, a variety of β-lactam structures have been discovered from bacterial cultures including *Streptomyces* species, which are known as cephamycin, cephabacin (cephem antibiotics), clavulanic acid (oxopenam antibiotic), thienamycin (carbapenem antibiotic), and sulfazecin (monobactam antibiotic).

The biosynthetic pathways of penam and cephem antibiotics are now well established and most of the enzymes involved in the biosynthesis of these β-lactams have been characterized biochemically. Initially, these antibiotics are made from three amino acids, α-aminoadipic acid, cystein and valine, by the action of L-α-aminoadipyl-L- cysteinyl-D-valine synthetase (ACV synthetase), a kind of nonribosomal peptide synthetase (NRPS). The first penam ring is subsequently formed from ACV tripeptide to isopenicillin N (IPN) with the help of IPN synthase (cyclase). In cephem producers, the penam ring is converted to cephem ring of deacetoxycephalosporanic acid (DAOC) by DAOC synthetase (expandase), after epimerization of IPN to penicillin N. The final modification and decoration steps of β-lactam rings are dependent upon their producing microorganisms, to give different β-lactam antibiotics.

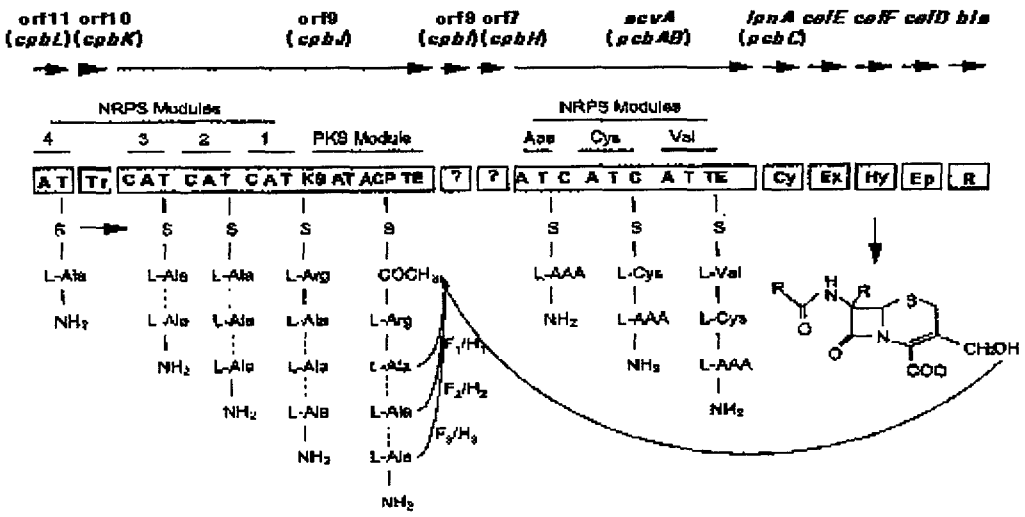
Among them, cephabacins are a new class of cephem antibiotics produced by Gram-negative bacilli, *Lysobacter lactamgenus* or *Xanthomonas lactamgena*. Different from other cephem antibiotics, cephabacins have oligopeptides at C-3 position of cephem ring. In addition, cephabacins are classified into F and H groups depending on the presence (F) or absence (H) of formylamino residue at C-7 position.

The gene cluster involved in the initial biosynthesis of cephabacin in *L. lactamgenus* has been cloned by a Japanese research group. They have already characterized the biochemical

properties of ACV synthetase, IPN synthase and DAOC synthase and other enzymes engaged in the early biosynthetic steps of cephabacin. However, neither the genes nor the enzymes responsible for the biosynthesis of oligopeptide moiety or formylamino residue have been identified or isolated.

Based on the chemical structure of cephabacins, it can be hypothesized that another NRPS system in addition to ACV synthetase is required for the introduction of oligopeptide moiety at C-3 position. By chromosomal walking, the upstream region of the known cephabacin biosynthetic gene cluster has been cloned using NRPS probes and sequenced. In the 24-kb upstream region, the genes for 4 NRPS region, 1 polyketide synthase (PKS) and 2 ABC transporters were deduced by comparison of sequence homology. Each biosynthetic gene has been subcloned and expressed in *Escherichia coli*, and the biochemical properties of the expressed proteins have been characterized.

Throughout this work, almost complete biosynthetic pathway of cephabacin has been elucidated. Based on the knowledge on cephabacin biosynthesis, the combinatorial biosynthesis of β-lactam antibiotics will be further attempted by gene disruption or replacement of biosynthetic genes in β-lactam producers.



The proposed biosynthetic pathaway of cephabacins
based on the organization of cephabacin biosynthetic gene cluster.

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