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Cloning of a beta-lactamase gene from Bacillus sp. J105

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Beta-lactamase is a type of enzyme responsible for bacterial resistance to penicillin-type antibiotics. Penicillin and related antibiotics have a common element in their molecular structure, a four-atom ring known as a beta lactam. The lactamase enzyme breaks that ring open, deactivating the molecule's antibiotic properties. We cloned a beta-lactamase gene from *Bacillus* sp. J105 with strong resistance against beta-lactamase antibiotics. The chromosomal DNA was isolated and partially digested with Sau3AI. 20-30 Kb of DNA fragments were selected and ligated into BamHI-treated pLAFR3. The recombinant plasmids were transferred into *E. coli* DH5a. Beta-lactamase positive clone was obtained and subcloned to 6.4 Kb of inserted DNA fragment with beta-lactamase gene. The gene was successfully expressed in *E. coli* DH5a. The expressed beta-lactamase was almost existed intracellular in the *E. coli*. Approximative 3~4% of the protein was secreted out of the *E. coli*. However, nearly all beta-lactamases were secreted out of *Bacillus*. It suggested that the secretion system is a difference between *Bacillus* and *E. coli*. The expressed beta-lactamase was purified from the *E. coli* using gel filtration. The molecular weight was estimated at 31 kDa in accord with the beta-lactamase from *Bacillus* sp. J105.