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NOTE

Immunochemical Reactivity of Polyclonal Antibody against Ampicillin Acylase of *Xanthomonas citri*

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Using the polyclonal antibody for *Xanthomonas citri* ampicillin acylase raised in *Pseudomonas*-free Balb/c mice, the immunochemical similarity of several types of penicillin acylases including *Erwinia aroideae* penicillin V acylase, *Escherichia coli* penicillin G acylase, *Pseudomonas melanogenum* and *Acetobacter turbidans* ampicillin acylases, and *Pseudomonas* cephalosporin acylase was examined. Among tested, only *P. melanogenum* ampicillin acylase showed the cross-reactivity with the antibody.

Penicillin acylases and cephalosporin acylases catalyze the hydrolysis of the acyl side chain of penicillin or cephalosporin antibiotics. This group of enzymes has been classified into 4 different categories based on their substrate preferences: penicillin G acylase, penicillin V acylase, ampicillin acylase and glutaryl-7-aminocephalosporanic acid (GL-7-ACA) acylase (14, 16, 18). Among them, ampicillin acylase has been extensively studied as an industrially important enzyme due to its ability to produce semisynthetic β-lactam antibiotics by acylating 6-aminopenicillanic acid (6-APA), 7-aminocephalosporanic acid (7-ACA) or 7-amino-3-deacetoxycephalosporanic acid (7-ADCA) with the acyl side chains (8, 19).

Recent advances in genetic engineering shifted the research focus to its gene cloning for overproduction and further site-directed mutagenesis for improvement of its reaction characteristics (3, 18). Thus, acylase genes from *Escherichia coli* (4), *Bacillus megaterium* (11), *Kluyvera citrophila* (1), *Arthrobacter viscosus* (12), *Proteus rettgeri* (2), *Bacillus sphaericus* (13), *Pseudomonas* sp. (9, 10) have been cloned and complete amino acid sequences of these enzymes have been deduced from nucleotide sequences. Even though amino acid sequences of penicillin G acylases from *E. coli* and *K. citrophila* show a strong homology (87%), no good similarity was found when comparing the primary structure of penicillin G acylase with that of penicillin V acylase or cephalosporin acylase. Furthermore, the secondary structures of penicillin acylases and cephalosporin acylases are not well known.

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In this study, the immunochemical analysis of penicillin acylases or cephalosporin acylases using the polyclonal antibody against *X. citri* ampicillin acylase was carried out for elucidating the similarity of their secondary structures, based on the fact that immunological study has been employed for the investigation of structural similarity of different protein sources.

Firstly, ampicillin acylase from *X. citri* IFO 3835 was purified in homogeneity, as reported previously (6). Penicillin G acylase from *E. coli* ATCC 11105 was purchased from Sigma Aldrich Co. (MO, U.S.A.), and GL-7-ACA acylase from *Pseudomonas* sp. SY-77-1 (crude enzyme) was kindly provided by Dr. Ki Hong Yoon, Chungkyung University, Taejon, Korea (17). The cell-free extract of *Erwinia aroideae* NRRL B-138 (21) was employed as penicillin V acylase. *Pseudomonas melanogenum* IFO 12020 (7) and *Acetobacter turbidans* ATCC 9325 (15) were also used as sources of ampicillin acylase after preparing cell-free extracts.

The purified ampicillin acylase of *X. citri* was dissolved in PBS buffer (140 mM sodium chloride, 2.7 mM potassium chloride, 1.5 mM potassium phosphate, monobasic, 8.1 mM sodium phosphate, dibasic), and mixed with the same volume of complete or incomplete Freund's adjuvant. The asceptically grown mice (Balb/c, *Pseudomonas*-free, supplied from KRIBB, Taejon, Korea) were immunized by boosting 0.25 ml suspension mixture intraperitoneally 3 times with 1 week intervals (5). The resulting antiserum (polyclonal antibody) was confirmed by enzyme-linked immunosorbent assay on microplate, using the polyclonal antibody as a primary antibody, rabbit anti-mouse IgG-alkaline phosphatase (AP) conjugate as a secondary antibody and nitroblue tetrazolium (NBT) with 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) as chromogen (20). In the examination of

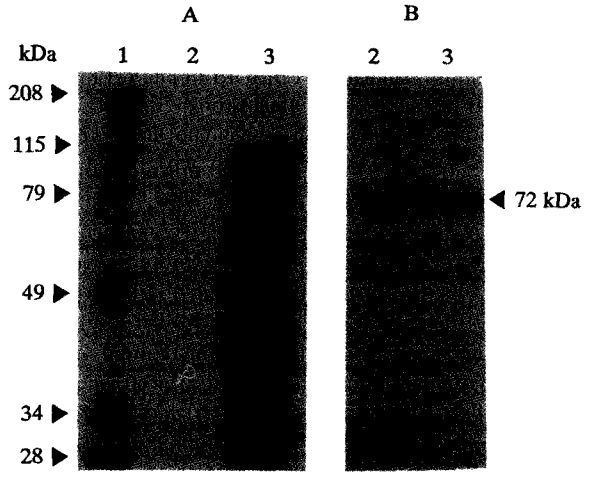

Fig. 1. Confirmation of polyclonal antibody by immunoblotting.
A, SDS-PAGE; B, immunoblotting with polyclonal antibody. Lane 1, molecular weight marker; lane 2, purified *X. citri* ampicillin acylase; lane 3, cell-free extract of *X. citri*.

immunochemical similarity, penicillin acylases and cephalsporin acylase were run on 0.7% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotted by the same system.

The titer of polyclonal antibody for *X. citri* ampicillin acylase was found to be 1 : 3,200. Considering that the polyclonal antibody could not be produced in non-asceptic commercial mouse (our result), this value is not so much low. The produced antiserum bound specifically with ampicillin acylase in cell lysates of *X. citri*, as seen in Fig. 1.

The immunochemical similarity of penicillin acylases and cephalosporin acylase was tested by blotting SDS-PAGE gels with the obtained antibody (Fig. 2). Among the ampicillin acylases tested, *P. melanogenum* enzyme showed an immunological reactivity with polyclonal antibody, whereas any strong signal on immunoblotting was not found in cell-free extract of *A. turbidans*. Furthermore, no immunochemical reactivity of polyclonal antibody with penicillin G acylase of *E. coli*, penicillin V acylase of *E. aeroideae* or GL-7-ACA acylase from *Pseudomonas* sp. was found in immunoblotting, as expected.

Based on the fact that *X. citri* ampicillin acylase is tetramer of the same subunit of 72 kDa (6) and *P. melanogenum* ampicillin acylase is homodimer of 72 kDa (7), it could be deduced that those enzymes have a lot of common features in their protein structure. However, it was interesting that there was no reaction of the polyclonal antibody with *A. turbidans* ampicillin acylase. It can be assumed that *A. turbidans* ampicillin acylase composed of heterodimer of 70 kDa and 72 kDa (15) have some similarity with penicillin acylases or cephalosporin acylase, which are known to have different α-subunit and β-subunit.

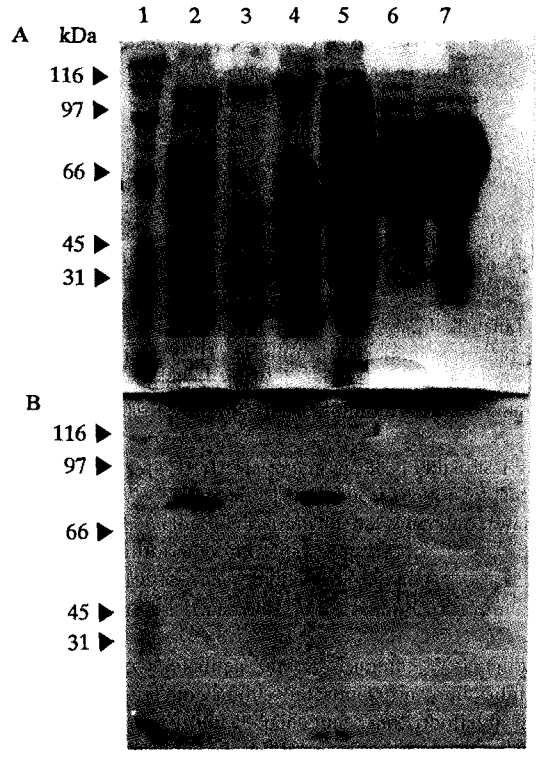

Fig. 2. Immunochemical binding of the polyclonal antibody with penicillin acylases and cephalosporin acylase.
A, SDS-PAGE; B, immunoblotting with polyclonal antibody. Lane 1, molecular weight marker; lane 2, cell-free extract of *X. citri*; lane 3, cell-free extract of *A. turbidans*; lane 4, cell-free extract of *P. melanogenum*; lane 5, cell-free extract of *E. aroideae*; lane 6, crude cephalosporin acylase of *Pseudomonas* sp.; lane 7, purified penicillin G acylase of *E. coli*.

In order to confirm our result, it is necessary to clone the genes for these enzymes and to compare their amino acid sequences.

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