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Production and Potential Uses of Monoclonal Antibodies to Pectate Lyases of *Erwinia chrysanthemi*

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Abstract

Specific monoclonal antibodies (MAbs) were produced against several of the five pectate lyase isozymes secreted by the enterobacterial phytopathogen *Erwinia chrysanthemi*, the agent of soft rot diseases. These characterized MAbs should be an interesting tool for immunoaffinity chromatography purification of pectate lyases and for further immunochemical, biochemical and cytological studies.

Keywords: Monoclonal antibodies, *Erwinia chrysanthemi*, pectate lyase, soft rot

Abbreviations: Monoclonal antibodies: MAbs; Pectate Lyase: PL; Sodium dodecyl sulfate: SDS; Polyacrylamide gel electrophoresis: PAGE; Isoelectric point: pI; Enzyme linked immuno-sorbent assay: ELISA.

1. Introduction

Erwinia chrysanthemi is the causal agent of soft rot on many plants, in crops and in conservation. The strain 3937 of *E. chrysanthemi*, pathogen to *Saintpaulia ionantha*, secretes in culture several inducible lytic enzymes in the presence of polygalacturonan: 5 pectate lyases (named PLa, b, c, d, e), 1 pectin methylesterase, 2 endoglucanases and 1 protease (1,8). The pectinolytic activities are found in extracts of infected plants (*Santpaulia*

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leaves and petioles, potato tubers and carrot roots) (2). The genes coding for these enzymes have been cloned and some have been mutagenized (4,5,6).

One of our objectives is to purify large amounts of these depolymerizing enzymes to study their parietal fixation in plants, their cytotoxicity and to determine their NH_2-terminal sequence by protein sequencing. So far, two pectate lyases have been purified by chromatofocusing, but the yield was low with this expensive technique (Bertheau, unpublished results). An other of our aims is to study *in planta* kinetics of enzymes appearance from the infecting bacteria. These two objectives could be achieved using labelled antibodies. In addition, these antibodies could be used to approach the study of protein secretion by *E. chrysanthemi*.

For these reasons, production of monoclonal antibodies (MAbs) has been initiated using the whole freeze dried culture supernatant (7) of the bacteria as immunizing antigen. This approach could furthermore result in antibodies against other antigens which have no enzymatic activity, but could be implicated in the phytopathogenicity of *E. chrysanthemi*.

2. Materials and Methods

Mice (BALB/c) were immunized by intraperitoneal injections of *E. chrysanthemi* culture supernatant. Spleen cells were removed and fused with non antibody-secreting murine myeloma (Sp2/O-Ag14) cells using polyethylene glycol (9). Hybridomas were selected according to the hypoxanthine-azaserine technique (9). Antibody-secreting hybridoma screening was done using an ELISA technique with the immunizing antigen. Fusion yield was very high, so hybridoma cultures were mixtures of clones and subsequent cloning was performed by the technique of limiting dilution (9) for six of the positive hybridomas.

E. chrysanthemi culture supernatant is a complex mixture visualized after electrofocusing by the enzymatic activity staining (1) or after SDS-PAGE by protein staining. Thus, immunoblots were performed after electrofocusing or SDS-PAGE using *E. chrysanthemi* culture supernatants or cell lysates of *E. coli* strains in which the *pel* genes had been cloned using plasmid vector pUC9 (6). Antigen-antibody reactions were visualized by an azo-dye technique.

3. Results

MAb10 and MAb11, secreted by two hybridoma clones, were characterized. MAb10 reacts with two antigenic bands with a similar mobility after electrofocusing to the PLd and PLe activities present in *E. chrysanthemi* culture

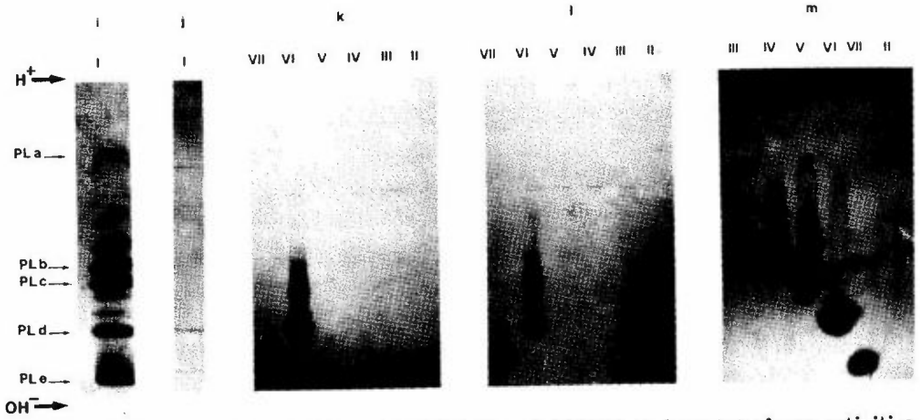

Figure 1. Immunoblots obtained with MAb10 and MAb11 and pectate lyase activities after electrofocusing.
Erwinia chrysanthemi **culture supernatant (I);** ***Escherichia coli*** **cell lysates from strains containing plasmid vector pUC9 (II) with *pelA* (III), *pelB* (IV), *pelC* (V), *pelD* (VI) and *pelE* (VII) genes. The samples have been focused in a 10% acrylamide gel containing Pharmacia 3–10 pH range ampholines (1). Pectate lyase activities (*i, m*) have been detected using a sandwich technique (1). Immunoblot of *E. chrysanthemi* supernatant was reacted with MAb10 (*j*) and immunoblots of cell lysates were reacted with MAb10 (*k*) or MAb11 (*l*). ⊔: localization of sample application.**

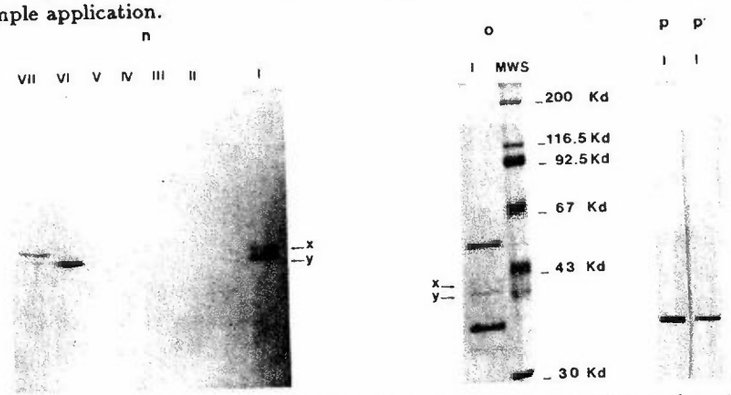

Figure 2. Immunoblots obtained with MAb11 and anti-PLb immune serum and protein profile after SDS-PAGE.
A 15% acrylamide SDS-PAGE containing *E. chrysanthemi* supernatant and *E. coli* cell lysates (legend Fig. 1) has been transferred on nitrocellulose. Immunoblot was reacted with MAb11 (n). Proteins of *E. chrysanthemi* supernatant and Molecular Weight Standard proteins (BIO-RAD) (MWS) have been stained with Coomassie Brilliant Blue R-250 after SDS-PAGE in a 11% acrylamide gel (o). From the same gel with the same antigen, immunoblots were reacted with anti-PLb immuneserum diluted 1/50 (*p*) and [1/500] (*p'*). The *x* and *y* arrows indicate the correspondence between the two antigenic bands and the protein bands.

supernatant (Fig. 1). This antibody, as well as MAb11, specifically reacts with cell lysates of *E. coli* carrying either *pelD* or *pelE* genes. The major band with which antibody reacts in each lysate has a similar pI as the single enzyme activity band of each of these lysates. The other bands revealed by the antibody which do not correspond to the activity band possibly result from protein degradation during cell lysis. On immunoblot after SDS-PAGE (Fig. 2), MAb11 reacts with antigenic bands present in the *E. chrysanthemi* supernatant. Each of these bands are present separately in *E. coli* carrying either *pelD* or *pelE* genes. Moreover, MAb11 reacts with an additional band of higher molecular weight in *E. coli* carrying *pelD* gene and does not react with the four other *E. coli* cell lysates. All of the 18 hybridoma clones resulting of three sequential cloning of two original positive hybridomas secrete antibodies specific for both PLd and PLe. We have not found so far a MAb certainly specific for only one of these pectate lyases.

In addition, 4 of the other positive hybridomas not cloned secrete antibodies against antigenic bands whose pI's correspond to those of PLb and PLc. However, detection during the cloning of MAbs specific for these pectate lyases has so far been unsuccessful. None of the isolated hybridomas has been shown to secrete detectable antibodies against PLa.

To obtain anti-PLb and anti-PLc secreting hybridomas, mice were immunized with isozymes PLb and PLc electroeluted from electrofocusing gels on which *E. chrysanthemi* culture supernatant had been resolved. Spleen cells wee removed and fused as described before. Using an ELISA technique and *E. chrysanthemi* culture supernatant antigen, we were unable to detect anti-PLb and PLc antibodies in either the immunsera or the supernatants of well-grown hybridoma cultures. After SDS-PAGE, immunoblots with the same antigen revealed that both immunsera and 3 of the hybridoma cultures contained antibodies to a single protein band (Fig. 2–p, p'). Such a similar molecular weight of PLb and PLc had been previously reported (3). These hybridomas are currently being cloned.

4. Discussion

MAbs were initially produced using the *E. chrysanthemi* culture supernatant. The antibody-secreting hybridomas obtained were expected to be of many different specificities, due to antigen complexity. So far, the first MAbs obtained were specific to PLd and PLe and we were unable to find the other specificities. This could be due to differences in immunogenicity of the different antigenic constituents or to a technical problem of detection.

Preliminary work to obtain specific MAbs to PLb and PLc seems to indicate a close immunochemical relationship between these isozymes, as well as it was demonstrated with PLd and PLe. A study of immunological relationships between these pectate lyases and those of other strains, other *Erwinia* or other pectinolytic microorganisms using a library of MAbs could be of interest.

The procedures used for producing MAbs against pectate lyases might possibly be useful for producing Mabs against pectate hydrolases, enzymes actually used in the industry.

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