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0273-2289/91/3101—0037$02.00

Immunological and Molecular Genetic Analysis of the Cellulase Component from *Penicillium funiculosum*

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Received February 1, 1991; Accepted March 18, 1991

ABSTRACT

Following immunization of rabbits, the antiserum was initially analyzed for antiendoglucanase activity using dot-blot ELISA methods. When compared with the preimmune serum, the antiserum showed strong response even at 25 ng concentration. The specificity of the polyclonal antibodies, raised against the partially purified endoglucanase component of *Penicillium funiculosum,* was determined by Oüchterlony double diffusion. Rocket electrophoresis confirmed the presence of only two types of antigens in the serum. The two rockets obtained were attributable to endo I and the merging of immunologically identical (but mobilitywise different) endo II and endo III. This antibody preparation was used as a probe. The deduced M1 of the cloned *E. coli* endo I was found to be 58 K_d by Western blotting.

Index Entries: *Penicillium funiculosum;* endoglucanase; antibodies.

INTRODUCTION

Various biotechnological industries concerned with the processes involving the conversion of plant raw material make extensive use of the yeast strain *Saccharomyces cerevisiae.* The availability of cellulase genes

makes it possible to construct yeast strains that secrete cellulases. Toward this goal, four different yeast strains producing a different *Trichoderma* cellulase have been constructed (*1*). The B glucanases/endoglucanases of the cellulase complex are commonly added at different stages of the brewing process to remove unwanted barley B glucans that may cause problems during filtration. These yeasts, secreting EGI, have been tested in pilot trials.

The filamentous fungus, *Penicillium funiculosum* (NCL isolate), produces a complete cellulase complex with activities comparable to those with *Trichoderma* cellulases. The aim of this project is to obtain yeast strain capable of expressing the endoglucanase gene of *P. funiculosum*. Indeed, we felt that, in order to clone some of these cellulase components, specific probes for the relevant component would be necessary. To this end, polyclonal antibody preparation has been produced to partially purified cellulase complex grown on a synthetic medium containing cellulose powder as the sole source of carbon (*2*). This report concludes that polyclonal antibody preparation is highly specific for endoglucanases produced by *P. funiculosum*. We have already reported the cloning of *P. funiculosum* cellulase gene and its expression in *E. coli* (*3*), where the sources of the cellulase gene was shown to be from *P. funiculosum* by using the polyclonal antibodies. Attempts are in progress to construct cellulolytic yeasts expressing *P. funiculosum* endoglucanases.

MATERIALS AND METHODS

Polyclonal Antibody Production to Endoglucanases

Antisera were prepared in New Zealand rabbits against partially purified cellulase enzymes from *P. funiculosum*. The antigen (2 mg) was injected sc. The booster doses were given every 15 d until sufficient antibody titer was obtained.

Dot-blot ELISA is a modification of the conventional ELISA and performed as described by Mawal et al (*4*). Rocket electrophoresis of partially purified cellulase preparation was performed as described in LKB Note No. 249 (March 1978) in 1% agarose gel containing 1:32 diluted antiserum in 24 m*M* sodium phosphate buffer (SPB), pH 7.0. The gel was run in the same buffer for 3–4 h at 20°C using 2 V/cm electric current. The cellulase activity in the gel was detected by incubating the dried immunological plate in contact with CMC agar plate at 50°C for 24 h. The cellulases were detected as clear zones in the turbid gels. Western immunoblot analysis using the polyclonal antibody was performed as described previously (*4*).

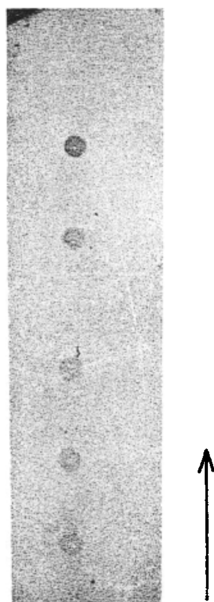

Fig. 1. Dot-blot ELISA of endoglucanses. Spots from bottom to top are of partially purified endoglucanase preparation in increasing amounts as 25, 100, 200, and 500 ng of proteins.

RESULTS

Polyclonal Antibodies to Endoglucanase

Following immunization of rabbits, serum was initially analyzed for antiendoglucanase activity using a dot-blot ELISA method. When compared with the preimmune serum, the antiserum yielded a strong response even at 25 ng concn. (Fig. 1).

In order to determine the specificity of the antiserum, the partially purified cellulase preparation before and after the BioGel purification served as antigens in the Oüchterlony double diffusion. Our results (data not shown) indicated a single precipitin line with purified endoglucanases, and two lines with partially purified cellulase preparation. The result indicated that the two lines are specific for the two types of immunologically distinct antigens corresponding to endoglucanases present in the partially purified preparation.

These results were further confirmed by Rocket electrophoresis. In Fig. 2, two rockets are seen, one small and one large. From the mobility of these rockets, in the agarose gel under electric field, it is clear that the smaller inner rocket is formed as a result of the endo I of 58 K_d protein

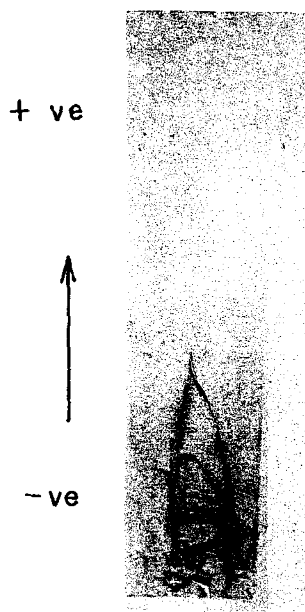

Fig. 2. Rocket electrophoresis of partially purified endoglucanase in 1% agarose containing 0.2% antisera. Electrophoresis was performed at room temperature at 50 V for 3–4 h in Na-Phosphate buffer, pH 7.0.

and the outer large rocket is formed as a result of the merging of the two antigenically identical (but mobilitywise different) endo II and III types. Further, we decided to use this as a probe for determining the mol wt of the cloned endo I in *E. coli* by Western blot analysis. The antibody preparation formed a single complex with purified *P. funiculosum* endo I and the *E. coli* endo I, indicating that the M1 of *E. coli* endo I is 58 K_d (not shown).

CONCLUSIONS

A highly specific antibody preparation to endoglucanases produced by *P. funiculosum* has been obtained. The endo I gene cloned in *E. coli*, along with the polyclonal antibody will provide a convenient marker of yeast expression systems. Polyclonal antibody preparation can be used to develop a single-step immunoaffinity method of purification of various endoglucanases from *P. funiculosum*.

ACKNOWLEDGMENT

I wish to thank Yogesh Mawal and Madhumalti Mawal for their timely suggestions and useful discussions during the course of this work.

REFERENCES

1. Knowles, J., Lehtovaara, P., Pentlila, M., Teeri, T., Harkki, A., and Salovuori, I. (1987), *Antonie Van Leeuwenhock* **53,** 335–341.
2. Sahasrabudhe, N. A., Lachke, A. L., and Ranjekar, P. K. (1987), *Biotechnol. Lett.* **9(2),** 881–886.
3. Sahasrabudhe, N. A. and Ranjekar, P. K. (1990), *FEMS Micro. Lett.* **66,** 291–294.
4. Mawal, M. R., Ranade, S. A., Mawal, Y. R., Ranadive, S. N., Bhattacharya, A., and Ranjekar, P. K. (1985), *Biosci. Rep.* **5,** 673–681.