Studies on Basidiomycete Cell-wall Lytic Enzymes

Shigekazu Yano

Basidiomycetes are valuable not only as food or feed, but also as microbial resources in several industrial fields, however, they have some disadvantages such as slow growth, susceptibility to fungal invasion, and so on. Hence, breeding with modern biotechnology has become significant, which requires efficient formation of reproducible protoplasts.

KA-prep, a culture filtrate of *Bacillus circulans* KA-304 grown on a cell-wall preparation of *Schizophyllum commune*, has an activity to form protoplasts from *S. commune* mycelia. KA-prep is superior to several known lytic enzymes in respect not only to the activity but also to reproduction frequency of the released protoplasts. Therefore, KA-prep has been expected to form protoplast from other basidiomycete as well as *S. commune*, which is useful for their molecular breeding.

The present study was undertaken firstly to establish an efficient enzyme system to prepare *Pleurotus ostreatus* protoplasts, in which KA-prep plays a leading role. Utilization of the protoplasts to a DNA-mediated transformation system was also investigated. Secondly, glycosidases of KA-prep were analyzed to obtain information for efficient protoplast formation, and were investigated from the viewpoint of their comparative biochemistry.

Chapter 1 revealed that KA-prep has an ability to form protoplasts from *P. ostreatus* mycelia, and that a few supplements of easily available enzyme preparations to KA-prep enhanced the protoplast-forming activity remarkably. The protoplasts thus formed were shown to be usable for the transformation system of *P. ostreatus.* This Chapter also suggested that KA-prep contains certain component(s) indispensable for the protoplast formation.

Chapter 2 describes a component of KA-prep necessary for protoplast formation from *S. commune* mycelia, which was isolated and identified as -1,3-glucanase. -1,3-Glucanase did not form protoplast by itself, however, it gave the protoplast-forming activity to the ammonium sulfate fraction of 30-50% saturation of KA-prep, which contained chitinase(s) and -glucanase(s) but was inactive in the protoplast formation. The result indicated that, besides -1,3-glucanse, chitinase(s) and/or -1,3- glucanse(s) are involved in the protoplast formation, which is discussed in Chapters 3 and 4. Cloning and expression of -1,3-glucanase gene was investigated for understanding and producing the enzyme.

Chapter 3 separated the ammonium sulfate fraction of 30-50% saturation of KA-prep into the chitinase preparation and the -glucanse preparation. The results are obtained that -1,3-glucanase and the chitinase preparation are the minimum requirements for the protoplast formation, and that the -glucanse preparation enhanced the protoplast formation in the mixture of -1,3-glucanase and the chitinase preparation. Chitinase I in the chitinase preparation was responsible to formation of *S. commune* protoplast in the presence of -1,3-glucanase. Comparative gene analysis of catalytic domain indicated that chitinase I is a family 19 chitinase, which is usually considered to degrade chitin in the cell-wall. A family 19 chitinase of *Streptomyces cyaneus* SP-27, which was effective as a supplement to the enzyme system for preparing *P. ostreatus* protoplast, was also investigated to confirm its role in the protoplast formation.

Chapter 4 deals with separation and characterization of β-glucanases in KA-prep. The -glucanases preparation, which was considered to play a secondary role in the protoplast formation in the preceding Chapter, contains at least four -glucanases (-glucanase I, II, III and IV), and -glucanase IV enhanced the protoplast-forming activity of the mixture of -1,3-glucanase and chitinase I.

The role of these enzymes in the protoplast formation was discussed, and suggestions were obtained for improving the protoplast formation.