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Characterization of Cell Wall Components and β-1, 3-glucanase of *Trimorphomyces papilionaceus*

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The chemical composition of the cell wall of *Trimorphomyces papilionaceus* 31 (monokaryotic cell), 313 (monokaryotic cell), 313-d (dikaryotic cell) was investigated. The cell wall was composed of neutral carbohydrates, amino sugars, proteins, and lipids.

Neutral carbohydrates were composed of glucose, galactose, mannose, arabinose, xylose, rhamnose and amino sugars were *N*-acetylglucosamine and *N*-acetylgalactosamine. The proportion of each component was different among the species. The polysaccharides could be distinguished on the basis of solubility; alkali-soluble fraction (FI), acid-soluble fraction (FII), alkali-, acid-insoluble fraction (FIII). In alkali-soluble fraction (FI) there were glucose, mannose, arabinose, rhamnose, and in acid-soluble fraction (FII), glucose, galactose, mannose, arabinose, xylose, rhamnose, and in alkali-, acid-insoluble fraction (FIII) glucose, galactose, mannose, arabinose, xylose, rhamnose. *N*-acetylglucosamine, *N*-acetylgalactosamine. The proportion of each component in each fraction was different among the species.

Partial hydrolysis of the complete cell wall produces β-1,3-glucan, β-1,4-glucan, β-1,6-glucan. The content of β-1,3-glucan was more than other components. To investigate the linkage type of the components, GC/MS was carried out. Although the linkage type was different among the species, there were main common linkage groups in each cell wall. Alkali-soluble glucan contained 1,4-arabinosyl, 1,6, 1,4-glucosyl linkages,

especially in *T. papilionaceus* 313 1,4-xylosyl linkage exists. Acid-soluble glucan mainly β-1,3-glucosyl linkages with some β-1,6-glucosyl and 1,4-arabinosyl linkages. Alkali, acid-insoluble glucan contains mainly 1,6-mannosyl linkages with some 1,4-arabinosyl linkages. The above results was reconfirmed by periodation and Smith degradation and I.r spectroscopy. Through the treatment of acid-soluble glucan with purified β-1,3-glucanase and analysis by methylation and NMR spectroscopy it was confirmed that there were β-1,3-linked-D-glucosyl residues a little branched with β-1, 6-linked-D-glucosyl residues.

Extracellular and intracellular cell wall hydrolyzing enzymes were purified from *T. papilionaceus* 31 through conventional procedures. It was found that they are β-1,3-glucanases. In purification steps of extracellular β-1,3-glucanases their affinity to Avicel enabled us to purify it through Avicel affinity chromatography and in purification of intracellular β-1,3-glucanase through buffer exchange to 20 mM pH 4.5 acetate buffer most other proteins were denatured and removed. It was very good step for the purification of the enzyme. The temperature optimum of both enzymes were 60°C, 55°C respectively and the pH optimum was 5.6 samely.

They are very heat-stable enzymes. The extracellular β-1,3-glucanase was able to hydrolyze laminarin, laminaribiose, CMC and cellobiose, and the intracellular enzyme was similar to the extracellular enzyme in ability of hydrolysis of several substrates except laminaribiose, CMC and cellobiose. The K_m values of the extracellular enzyme to laminarin and pNPG were 1 mg/ml and 26 mM, respectively. In the

intracellular enzyme the values were 3.7 mg/ml, 0.3 mM, respectively. The extracellular enzyme was inhibited by copper and zinc ions and the intracellular enzyme was inhibited by copper, magnesium and mercuric ions. The N-terminal sequence of the extracellular enzyme was X-Val-Asn-Val-Gly-Trp-X-Tyr-Gly-Ser-Gln-X-Ile-Leu-X-Val-Asn-Leu-Gly and the N-terminal of the intracellular enzyme was blocked.

It was found that the extracellular enzyme had no homology with the intracellular enzyme *Saccharomyces cerevisiae*, but had homology with *Bacillus circulans, Trichoderma koningii* in Western blotting. The extracellular enzyme hydrolyzed laminarin and laminarioligosaccharides at random in the early stage of reaction but in course of the reaction the products were changed and finally larger amounts of laminaribiose were generated. The intracellular enzyme hydrolyzed laminarin and laminarioligosaccharides by the similar pattern to the extracellular enzyme but finally glucose was generated. And so by the results it was found that they have transglycosylation activity.

The possibility of binding of the extracellular enzyme to the cell wall was ascertained by flow cytometer (FACS) and indirect fluorescent antibody staining and Confocal microscopy. It was found that the enzyme was bound to the cell surface, but the region was not restricted to specific sites.