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Aspergillus 속 균주 유래 효소를 이용한 Gypneoside V의 ginsenoside Rg3로의 전환

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Bioconverstion of Gypenoside to Minor Ginsenoside Using Purified Enzyme from *Aspergillus sp.*

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Objectives

The gypenosides exist mainly as dammarane type glycoside and are important secondary metabolites in *G. pentaphyllum*. The structure of gypenosides is closely resemblesd to that of ginseng saponin (ginsenoside). The Gypenoside V is similar to the ginsenoside Rb1 and correspondingly it belongs to the major protopanaxadiol gypenosides. In this study, gypenoside V was transformed to ginseng saponins by the purified enzymes which were extracted from culture broth of Lactic acid bactrium. Gypenoside hydroryzing glucosidase was purified from *Aspergillus sp.* by combination of IEX, Gel filtration, HIC, CHT hydroxyapatite column chromatography.

Materials and Methods

○ Materials

Gypenoside V and minor ginsenosides were obtained from Ginseng Genetic Resource Bank, Yong-In, Korea.

○ Methods

ginsenoside-hydrolyzing-enzyme was purified from *Aspergillus sp.* by Q sepharose HR, sephacryl S300 HR, phenyl-sepharose HR, and CHT (ceramic hydroxyapatite) column chromatography. The following buffers were used: buffer A, 10 mM sodium phosphate buffer (pH 7.0); buffer B, 20 mM sodium phosphate buffer containing 1M NaCl (pH 7.0); buffer C, sodium phosphate buffer containing 1M ammonium sulfate; buffer D, 500 mM potassium phosphate buffer (pH 6.8).

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Results

The purified enzyme was monomeric protein with a molecular mass estimated be 110kDa. This is the first report on the enzymatic conversion of gypenosides into minor ginsenosides such as ginsenoside C-K and Rg3 which are sources of multifarious and beneficial saponins. The study of gypenoside V hydrolysis by the purified enzyme showed that it converted gypenoside to minor ginsenoside Rg3 quite efficiently. A total 82.1% of gypenoside V, with an initial concentration of 10000ppm, was converted into Rg3 in 24 h by the enzyme at 37 ℃and pH 7. The pathway was observed to be gypenoside V to Rg3, which indicates that the enzyme hydrolyzes the one glucose linked with rhamnose molecules at C-20 of ginsenoside gypenoside V.

Table 1. The enzyme purification.

Purification steps	Protein(mg)[a]	Total activity(U)[b]	Specific activity(U/mg)	Purification(fold)
Crude extract	2618.81	112.17	0.043	1.0
35-40% $(NH_4)SO_4$	231.94	33.54	0.145	3.4
Q-Sepharose	26.61	23.33	0.877	20.5
Phenyl-Sepharose	8.01	16.7	2.085	48.7
Sephacryl S200	4.71	13.11	2.783	65.0
CHT	2.46	9.18	3.732	87.1

[a] Protein contents were determined accroding to the method of bradford, using bovine serum albumin as the standard

[b] One unit(U) of b-D-glucosidase was defined as the amount of enzyme liberaring 1nmol/min of p-nitrophenyl

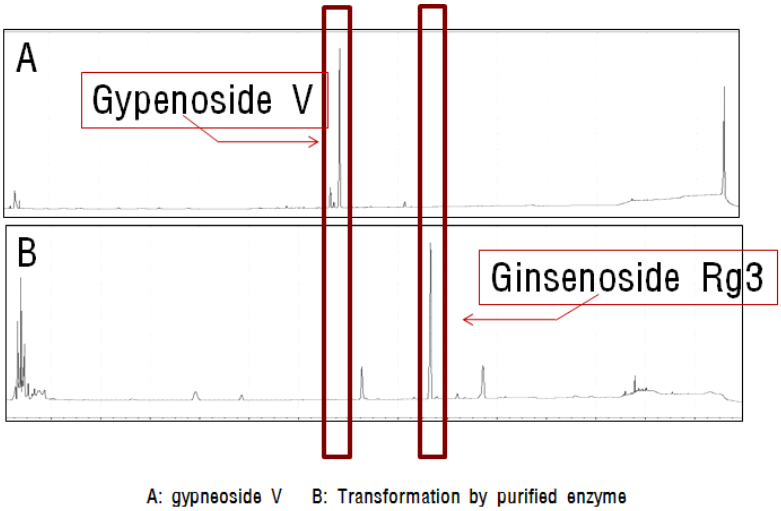

Fig. 1. The products of ginsenoside Rg3 by purified enzyme in HPLC