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Development of New Biohealth Products

Dong Seok Lee

Biohealth Products Research Center supported by MOST & KOSEF and
Dept. of Medical Laboratory Science, College of Biomedical Science
and Engineering, Inje University, Gimhae, 621-749, Korea

I. The oligosaccharide fraction AIP1 purified from *Artemisia iwayomogi* was shown to increase the population of immune cells *in vitro* and to have the immunomodulating and anti-tumor activities. In this study, the effects of the AIP1 oligosaccharide on murine macrophages and of the crude AIP polysaccharide on diabetic rat and lactic acid bacteria were examined. To determine how the AIP1 presents its activity, we have studied the effect of the fraction on the mouse peritoneal macrophages *in vitro*. To determine how the AIP1 fraction affects survival of macrophages, the degrees of apoptotic death of the cells in culture were determined using DNA fragmentation assay. The macrophage cultures supplemented with the AIP1 fraction show significant decreases in the apoptotic cell death than those of the control cultures, indicating the immunomodulating effect of the fraction might come from delaying or suppressing death of the macrophage type cells. Effect of crude AIP polysaccharide on diabetic rat was studied by feeding the extract to streptozocin (STZ)-induced male Sprague-Dawley diabetic rats. The diabetic animals were fed a basal diet of kcal of carbohydrate:protein:lipid = 60:20:20 supplemented with 100ml/kg/day of AIP extract for 5 days. The animals fed the diet supplemented with drinking water were employed as a control group. Changes (Δ) in serum glucose, triglyceride and total cholesterol levels were measured before and after the feeding period. The Δs-glucose, Δs-triglyceride and Δs-total cholesterol levels in the AIP treated group were -52.4 ± 9.4 ㎎/dℓ, -100.0 ± 43.6 ㎎/dℓ and

-214.0±32.7 ㎎/dℓ respectively ($p<0.05$), indicating that oral administration of AIP extract shows hypoglycemic, hypotriglyceridemic and hypocholesterolemic activity in diabetic rats. Additions of AIP extract to the cultures of *Bifidobacterium* and *Lactobacillus* were also shown to stimulate their growth and lactic acid production.

Ⅱ. Endo-β-1,3-1,4-glucanase hydrolyzes the mixed, linked (1,3-1,4)-β-glucans that constitute the major part of the endosperm cell walls of cereals such as barley and oat. Endo-β-1,3-1,4-glucanase cleaves β-1,4 linkages adjacent to β-1,3 bonds in barley β-glucan or lichenan, yielding oligosaccharides including chiefly 3-*O*-β-cellobiosyl-D-glucose and 3-*O*-β-cellotriosyl-D-glucose. A gene coding for the endo-β-1,3-1,4-glucanase of *B. circulans* ATCC21367 was cloned into *Escherichia coli*. The cloned enzyme hydrolyzed barley β-glucan or lichenan to produce oligosaccharides. The molecular mass of the enzyme was 28 kDa and remained within the cytoplasm of *E. coli*. A 771 bp open reading frame was observed in the 2.0 kb *Pst* I fragment of the recombinant plasmid pLL200K. The deduced protein sequence consists of 257 amino acids and has a putative signal peptide of 26 amino acids. The amino acid sequence of the endo-β-1,3-1,4-glucanase showed 68, 51% homology to that of the previously reported endo-β-1,3-1,4-glucanase from *Bacillus* strain N-137 and *B. brevis*, respectively. Obtaining oligosaccharides efficiently from barley using the cloned endo-β-1,3-1,4-glucanase is very attractive for the development of new biohealth products.

Key word : polysaccaride, oligosaccharide, *Artemisia iwayomogi*, macrophage, diabetic rat, lactic acid bacteria, *Bacillus circulans*, endo-β-1,3-1,4-glucanase, β-glucan