특별강연 3

담자균을 이용한 기능성 쌀의 개발

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Development of Functional Rice by Use of Basidiomycetes

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Mushroom as functional foods

In nature, basidiomycetes is one of the most powerful "decomposer", especially in the forest. This has, of course, a positive as well as negative effects to man.

Nowadays, however, man makes use of this microorganism in various ways. First of all, we obtain a very valuable food, mushrooms, from the organism. Throughout the world about 10,000 species of basidiomycetes are identified out of 22,000 species that are estimated to be present. At present 30-40 species are commercially cultivated. This is one of the big agricultural crops.

Another use of basidiomycetes is the production of biologically active products mainly by the submerged tank culture process. Various mushrooms are known to have various biological activities such as anticancer, antiviral, antibacterial, blood pressure reducing, blood sugar reducing, cholesteral reducing and antithrombosis activities. The more familiar examples are such as Krestin (*Coriolus versicolor*), Lentinan(*Lentinus edodes*), Schizophillan (*Schizophyllum commune*), Coplang(*Coriolus versicolor*), and so on. Recently the Ganoderma, Cordyceps, Phellinus and Agaricus mushrooms are getting more and more popularity based on their biological activities, especially the anticancer activity.

The utilization of basidiomycetal strains for the solid state fermentation of cereal was attempted in Functional Foods Lab. of Yeungnam University. This is a new approach for the exploitation of the biological activities of basidiomycetes.

Solid substrate fermentation(SSF)

According to E. Cannel solid state fermentation refers to the growth of micro-organisms on solid materials without the presence of free liquid. While the presence of moisture is necessary in solid fermentation, it exists in an absorbed or complex form within the solid matrix. Solid state fermentation does not refer to the fermentation of solid substrates in a liquid media nor does it refer to the fermentation of "slurries"(i.e. liquids with high levels of insoluble solids).

Regarding to the characteristics of SSF Hesseltine illustrated the advantages and disadvantages of the process in detail. Some of the more important points are.

(1) The medium is relatively simple since a single whole grain plus water is needed.

(2) It is apparent from the work reported that the process may be scaled up either as batch or continuous fermentations.

(3) The required space occupied by the fermentation equipment is small relative to yield of product because less water is used and the substrate is concentrated.

(4) The low moisture required to get maximum yields of the product with fungi excludes or reduces greatly the problem of bacterial contamination.

(5) Since less substrate is used to produce the desired product, less solvent is required to extract the product. There are no enormous amounts of liquid waste as in liquid fermentations to present a disposal treatment problem. In fact, there is no liquid to be disposed of.

(6) Since the product is concentrated in the solid substrate, it may be dried and incorporated directly into animal feed at less cost because less moisture must be removed.

Problem with solid substrate fermentations become obvious when one works with several types of mould fermentations.

(1) The types of micro-organisms are limited to those which can grow at reduced moisture

levels, namely, fungi, some yeasts, and Streptomyces.

(2) In a large scale operation, the heat generated by respiring microorganisms must be removed. This can be more difficult in SSF.

(3) Using monitoring devices to determine moisture, pH, free oxygen and carbon dioxide, and product yield becomes a real problem.

Functional Rice

There are already many works and informations on the various biological activities of the various basidiomycetal fruit body. The main idea of the SSF of cereals with basidiomycetes is to produce the cereals that are cultured with basidiomycetal strains and thereby acquired the biological functions that are originated from the basidiomycetes. Cereals, including soybean, were pretreated by soaking and inoculated with selected strains of basidiomycetes.

The inoculated cereals were then incubated at the adequate temperature for the strains until the whole mass was covered with mycelium. These moldy cereals are named as basidio-rice, Basidio-wheat, etc. These basidio-cereals could be further processed to various functional products such as functional rice porridge, snacks and so on.

The biological activities of the products were evaluated *in vitro* as well as *in vivo* experiments. Some of the noteworth results are demonstrated below.

To begin with, the safety of the product as food was examined. In toxicity test by use of mouse, the polysaccharides did not show any symptoms of toxicity up to 1000㎎/㎏ body weight.

The basidiomycetal polysaccharides did not show any cytotoxicity against the cancer cell in *in-vitro* test.

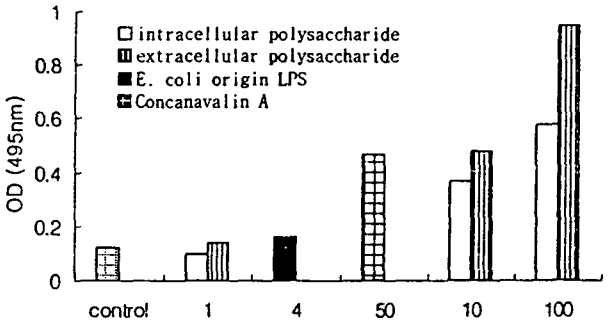


Fig. 1. Effect of intra and extracellular poly- saccharide from *P. igniarius* on the proliferation of BALB/c spleen cells.

Spleen cells were cultured with extra and intracellular polysaccharides originated from *P.igniarius* for 2 days.

Proliferation was determined by MTT assay.

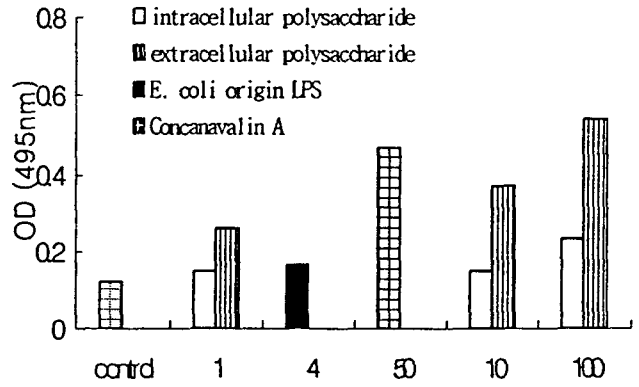
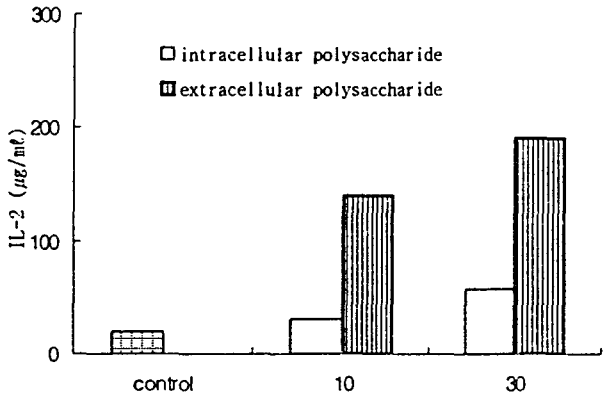


Fig. 2. Effect of intra and extracellular poly- saccharide from *A.cylindracea* on the proliferation of BALB/c spleen cells.

Spleen cells were cultured with extra and intracellular polysaccharides originated from *A. cyclindracea* of for 2 days.

Proliferation was determined by MTT assay.

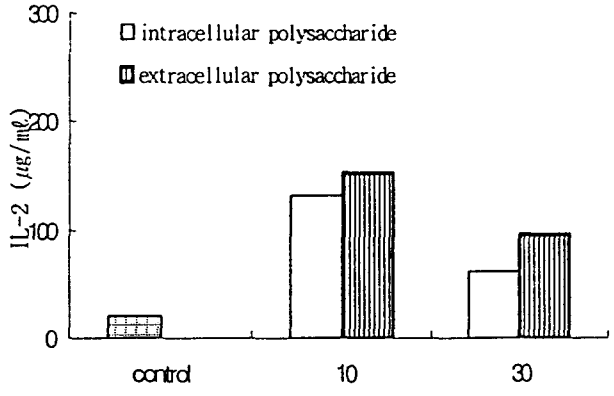
The intracellular and extracellular polysaccharides of *P. igniarius* as well as *A. cylindracea* increased the IL-2 considerably.



Concentration of polysaccharides (μg/ml)

Fig. 3. Effect of intra and extracellular polysaccharide from *P. igniarius* on expression of Interleukin-2 receptors on BALB/c spleen cells.

The cells were incubated with various concentrations of *P.igniarius* polysaccharides for 48hrs at 5% CO_2 incubator.



Concentration of polysaccharides (μg/ml)

Fig. 4. Effect of intra and extracellular poly- saccharide from *A. cylindracea* on expression of Interleukin-2 receptors on BALB/c spleen cells.

The cells were incubated with various concentrations of *A.cylindracea* polysaccharides for 48hrs at 5% CO_2 incubator.

The mice implanted with sarcoma 180 was orally fed with polysaccharides extracted from the soybean cultured with *P.igniarius*. In 30 days of observation the treated group showed life span extension of 64% compared to the control group.

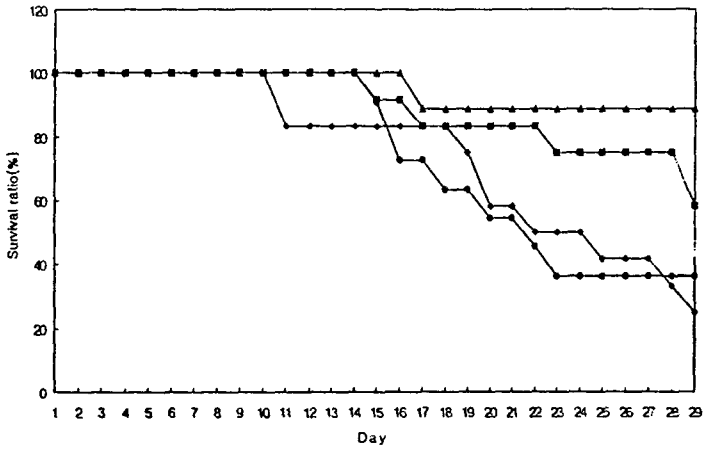


Fig. 5. Survival rate of mouse implanted with the Sarcoma-180 tumor cell at the peritoneal

-◆- Control

-■- Exts of soybean(50mg/kg)

-▲- Exts of Soybean cultured with *P.igniarius* (50mg/kg)

-●- Exts of *P. igniarius* fruit body(25mg/kg)

The liver protecting activity of the methanol extract from the functional rice cultured with P. igniarius was examined in vivo by use of rats. The rats of test group were fed with the methanol extract of the functional rice in addition to the regular diet while the rats of control group were fed with the regular diet only. After 4 weeks of feeding, all the groups were treated with CCl_4 at the dosage of 0.1 ml/100g body weight in 3 consecutive days. The various biochemical tests were conducted and the electron microscopic observations on the cell damage were made.

Table 1. Effect of extracts of waxy brown rice fermented with Basidiomycetes on the body weight gain in rats

Group	Body Weight(g)		
	Initial Weight	Final Weight	Daily increase
N	136.8 ± 3.7^{a}	260.2 ± 7.7^{a}	4.41 ± 0.15^{a}
NC	136.1 ± 3.7^{a}	241.6 ± 2.5^{de}	3.77 ± 0.17^{c}
NCB	136.5 ± 4.4^{a}	249.5 ± 8.5^{b}	4.03 ± 0.34^{bc}
NCP	135.9 ± 6.7^{a}	249.3 ± 8.1^{b}	4.05 ± 0.42^{bc}

The values are mean±S.D (n=7)

The values followed by the same letter are not significantly different ($P<0.05$)

N : Diet group (control)

NC : Treated with CCl_4 only

NCB : Fed with methanol extract of waxy brown rice, followed by CCl_4 treatment

NCP : Fed with methanol extract of waxy brown rice fermented with *Phellinus igniarius*, followed by CCl_4 treatment

Table 2. The effect of extracts of waxy brown rice fermented with Basidiomycetes on the internal organs weight gain in rats

Group	Relative Weight(%)				
	Liver	Kidney	Spleen	Lung	Heart
N	2.80±0.36^{d}	0.71±0.04^{b}	0.18±0.02^{b}	0.49±0.05^{a}	0.34±0.04^{b}
NC	3.92±0.40^{a}	0.81±0.07^{a}	0.22±0.02^{a}	0.52±0.05^{a}	0.38±0.04^{a}
NCB	3.90±0.34^{a}	0.74±0.04^{b}	0.19±0.01^{b}	0.51±0.05^{a}	0.37±0.03^{ab}
NCP	3.42±0.20^{bc}	0.75±0.03^{b}	0.18±0.02^{b}	0.50±0.06^{a}	0.35±0.03^{ab}

The values are mean±S.D (n=7)

The values followed by the same letter are not significantly different (P<0.05)

Table 3. The effect of each sample on the serum glutamic oxaloacetic transaminase(GOT) activity in rats

Group	Glutamic oxaloacetic transaminase	
	activity(karmen unit/ml)	Inhibition(%)
N	223.2±12.8^{f}	
NC	1151.1±110.6^{a}	
NCB	870.4±194.4^{b}	24.4
NCP	647.7±21.2^{c}	43.7

The values are mean±S.D (n=7)

The values followed by the same letter are not significantly different (P<0.05)

Table 4. The effect on the serum glutamic pyruvic transaminase(GPT) activity in rats

Group	Glutamic pyruvic tranaminase	
	activity(karmen unit/㎖)	Inhibition(%)
N	88.4±15.2[c]	
NC	946.8±67.1[a]	
NCB	783.5±62.4[b]	17.2
NCP	446.8±13.8[c]	52.8

The values are mean±S.D (n=7)

The values followed by the same letter are not significantly different (P<0.05)

Table 5. The effect on the serum alkaline phosphotase (ALP) activity in rats

Group	Alkaline phosphotase	
	activity(K-A unit)	Inhibition(%)
N	27.0±9.8[c]	
NC	118.1±30.1[a]	
NCB	103.3±22.2[a]	12.5
NCP	56.3±9.9[b]	52.3

The values are mean±S.D (n=7)

The values followed by the same letter are not significantly different (P<0.05)

Table 6. The effect on the serum lactate dehydrogenase(LDH) activity in rats

Group	Lactate dehydrogenase	
	activity($\times 10^2$) (Wroblewski unit)	Inhibition(%)
N	11.53 ± 0.67^{b}	
NC	13.24 ± 1.17^{a}	
NCB	12.58 ± 0.73^{ab}	5.0
NCP	9.39 ± 0.1^{cd}	29.1

The values are mean±S.D (n=7)

The values followed by the same letter are not significantly different (P<0.05)

Table 7. The effect on the serum γ - glutamyl transpeptidase(γ -GTP) activity in rats

Group	γ -glutamyl transpeptidase	
	activity(mU/ml)	Inhibition(%)
N	65.3 ± 6.4^{d}	
NC	322.3 ± 13.0^{a}	
NCB	183.6 ± 20.0^{b}	43.0
NCP	98.6 ± 21.5^{cd}	69.4

The values are mean±S.D (n=7)

The values followed by the same letter are not significantly different (P<0.05)

Table 8. The effect on the serum albumin content in rats

Group	Albunim	
	serum content(g/dl)	Decrease(%)
N	4.61±0.44[a]	
NC	3.28±0.31[d]	28.9
NCB	3.73±0.31[c]	19.1
NCP	4.14±0.16[b]	10.2

The values are mean±S.D (n=7)

The values followed by the same letter are not significantly different (P<0.05)

Table 9. The effect on the serum total protein content in rats

Group	Total protein	
	serum content (g/dl)	Decrease(%)
N	3.99±0.29[a]	
NC	2.61±0.18[d]	34.6
NCB	3.00±0.11[c]	24.8
NCP	3.30±0.23[b]	17.3

The values are mean±S.D (n=7)

The values followed by the same letter are not significantly different (P<0.05)

Table 10. The effect on the serum total billilubin contents in rats

Group	Total bililubin(mg/dl)	
	serum content(mg/dl)	Decrease(%)
N	0.97 ± 0.20^{bcd}	
NC	1.86 ± 0.36^{a}	
NCB	1.36 ± 0.71^{b}	26.9
NCP	0.80 ± 0.09^{cd}	57.0

The values are mean±S.D (n=7)

The values followed by the same letter are not significantly different (P<0.05)

Table 11. The effect on the serum direct billilubin contents in rats

Group	Direct bililubin	
	serum content (mg/dl)	Decrease(%)
N	0.75 ± 0.26^{cd}	
NC	1.54 ± 0.15^{a}	
NCB	1.19 ± 0.35^{ab}	22.7
NCP	0.93 ± 0.34^{bcd}	39.6

The values are mean±S.D (n=7)

The values followed by the same letter are not significantly different (P<0.05)