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사탕수수 찌꺼기를 이용한 여름느타리 발효부산물의 생물활성

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BIOLOGICAL ACTIVITIES OF PRODUCTS FROM SUGAR CANE BAGASSE FERMENTATION BY *Pleurotus sajor-caju*

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ABSTRACT : In order to evaluate the biological activities of some fractions from the fungal(*Pleurotus sajor-caju*) fermentation products of sugar cane bagasses, the antimutagenicity, the glyceollin elicitor activity on soybean and the effect on the stem elongation in pea were observed. The alcohol extract fraction and DMSO soluble fraction had excellent antimutagenicity even though it is weaker than that of the extracts from the fruiting bodies. All of the extracts had the ability to elicit glyceollins in soybean cotyledons and these extracts could be helpful for plants to protect themselves from pathogenic contaminations. IAA and the extracts had shown synergistic effects on pea stem elongation in all experimental groups positively determined.

Keywords : *Pleurotus sajor-caju*, sugar cane bagasse, antimutagenicity, glyceollin, stem elongation,

Introduction

A large amount of cellulosic agricultural wastes generated all over the world have not been used effectively, so far. Conversions of such biomass is poor at present, and utilization is inefficient, because of physico-chemical barriers to biological degradation and/or anti-quality components such as toxicants that restrict biological usages. Many efforts have been made to recycle and reuse these wastes as profitable materials by enzymatic hydrolysis[1], which turned out quite difficult to be applied for the practical uses, because its hydrolytic efficiency was very low. Nowadays, the use of desired microorganisms seems to be promising to degrade and recycle cellulosic agricultural wastes as 1) the animal feed[2] ; In the last years the necessity of additional feed reserves especially for ruminants demanded efforts to increase the digestibility of rice straw and analogous cellulosic wastes by several methods. Agricultural residues contain considerable quantities of cellulose and hemicellulose that can be excellent energy sources in feed for ruminants, 2) the substrate for edible mushroom production[3,4]; The presence of lignin is one of the limiting factors for a rational use of cellulosic wastes and some of edible mushrooms are examplary organisms capable of degrading cellulosic wastes. And we have studied to find 3) some bioactive materials from the fermentation products of sugar cane bagasses by *Pleurotus sajor-caju*. The antimutagenicity, the glyceollin elicitor activity on soybean and the effect on the stem elongation in pea were observed.

Materials and Methods

Fermentation and extraction

Sugar cane bagasses from Okinawa prefecture(Japan) were irradiated at the dose of 25kGy by gamma-ray source(Co-60) and fermented on the Mandel' s media with the addition of 5% rice bran by *Pleurotus sajor-caju*. Two hundreds grams of each byproducts was extracted with water at 100℃ for 4hr as a water soluble fraction and with 85% ethyl alcohol(Et-OH) at 80℃ for 3hr thrice as a alcohol extract fraction and dimethylsulfoxide(DMSO) soluble fraction. Both fractions were concentrated in the vacuum evaporator, resuspended into 50㎖ of each solvents, sterilized by Millipore filter of 0.45 ㎛ porosity and stored at 4℃ for futher experiments.

Ames test

Antimutagenicity test was done by the method of Ames et al.[5]. *Salmonella typhymurium TA100* and *TA98* freezed with 0.09% of DMSO (1.0-2.0 X 109 cells/㎖) were thawed and resuspended with 1/15M phosphate buffer after centrifugation at 3,000 x g for 15min. Aflatoxin B1 with S9 fraction and furylfuramide(AF2) were used for the stimulation of back mutation. 100㎕ of samples and strains respectively were added to the test tube of top agar (2.5㎖ at 45-47℃, 0.6% of agar and 0.6% of NaCl). However, 0.5㎖ of

S9 mixture was added and the whole mixture were preincubated at 37℃ for 20min only for the aflatoxin B1. After quick mixing between palms, this mixture was poured onto the base agar, and spread it homogeneously by tilting and swirling. The cover of Petri dish(sterilized by γ-ray) was put on quickly protecting from light effect. Incubated at 37℃ for 48hr in the dark incubator.

Cotyledon assay

For the cotyledon assay[6], the surface of soybean was sterilized with 10% $NaClO_3$ for 10min, washed by distilled water and germinated at the vermiculite bed for 7days at 25 ℃ in the dark. The surface of soybean cotyledon was sterilized with 10% $NaClO_3$ for 5min, washed by distilled water and cut at the dimension of ca. 7mm x 5mm x 2mm with dissecting knife. After administration of extracts, cotyledons including 2㎖ of assay buffer(4mM CH_3COONa, 3mM Na_2CO_3) were incubated at 26℃ for 24hr in the dark with enough humidity and extracted with 80㎖ of 80% ethyl alcohol overnight at 4℃. The extract was concentrated by vacuum evaporator after filtration by Molcut (Millipore Ltd., exclusion limit = 10,000). The peak of glyceollin was analysed by HPLC(Inertsil ODS-3 column, Waters TM486 Tunable Absorbance detector(280㎚), isocratic elution ; H_2O:CH_3CN=57:43, V/V, JASCO 880-PU Intelligent HPLC pump)[7].

Assay of indole acetic acid(IAA)-induced growth

For the IAA assay[8], pea seeds were washed in running tap water for 24hr and germinated at 25℃ in the dark. On the day7 after planting, one segment(1㎝ long) was cut just below the hook that had 2-3㎝ long third internode. Twenty segments were weighed and placed in Petri dishes containing 10㎖ of 1mM IAA and 100㎕ of samples. The segments were dried on filter paper and weighed again after 3hr at 25℃ in the dark. The percentage inhibition was calculated by the following formula. Percent inhibition = 100 x (C-T)/C where, C = increase in fresh weight of the control, T = increase in fresh weight of the treated sample.

Results and Discussion

Antitumor effects

The occurrence of the back mutation with AF2 of TA100 was 2,136±465.1cfu(colony forming unit)/plate and that of TA98 was 131.7±40.5cfu/plate. The water extract fraction, alcohol extract fraction and alcohol extract fraction soluble in dimethyl sulfoxide(DMSO) suppressed the back mutation by AF2 suggesting antimutagenicity ; 63.6%, 70.3% and 78.5% in TA100 and -6.7%, 95.6% and 45.5% in TA98, respectively(Table 1). From the tests which back mutation was stimulated by aflatoxin B_1, the water extract fraction did not have any antimutagenicity, but alcohol extract fraction and DMSO soluble fraction suppressed back mutation 28.7% and 37.2% in TA100 and 49.2% and 80.9% in TA98, respectively(Table 2). When Zhuang et al.[9] tested antitumor activity of the extracts from the fruiting body of *P. sajor-caju*, they found alcohol extract fraction had 86.4% of tumor inhibition activity against Sarcoma 180 tumor cells. It could be thought that the alcohol extract fraction and DMSO soluble fraction had excellent antimutagenicity even though it is weaker than that of the extracts from the fruiting bodies. It is necessary to analyse more whether the same molecules come from both fermentation byproducts and fruiting body or not.

Table 1. Effects on *Salmonella typhymurium TA100* and *TA98* of which back mutation initiated by AF2

	TA100		TA98	
	cfu/plate	%inhibition of mutation	cfu/plate	%inhibition of mutation
control	2,136.0 ± 465.1	0.0	131.7 ± 40.5	0.0
water extract	777.3 ± 76.6	63.6*	194.0 ± 62.0	-6.7
Et-OH extract	634.7 ± 40.8	70.3*	8.0 ± 3.3	95.6**
DMSO soluble alcohol extract	459.0 ± 105.0	78.5*	99.0 ± 20.6	45.5*

Values represent mean ± SD of revertants per plate triplicately.
* : p<0.05, ** : P<0.01.

Table 2. Effects on *Salmonella typhymurium TA100* and *TA98* of which back mutation initiated by Aflatoxin B_1

	TA100		TA98	
	cfu/plate	%inhibition of mutation	cfu/plate	%inhibition of mutation
control	381.3 ± 23.2	0.0	849.3 ± 104.6	0.0
water extract	497.3 ± 51.1	-30.4	908.0 ± 94.5	-6.9
Et-OH extract	271.7 ± 31.5	28.7	431.3 ± 115.0	49.2*
DMSO soluble alcohol extract	239.3 ± 62.8	37.2	162.0 ± 29.0	80.9**

Values represent mean ± SD of revertants per plate triplicately.
* : p<0.05, ** : P<0.01.

Induction of glyceollin

The result of glyceollin analysis by HPLC after cotyledon assay from the fermentation byproducts was shown in Table 3. The amount of glyceollin of the Et-OH extract was more than that of the other experimental groups totally. In the Et-OH extract group of fraction No.1 and 2 with the administration of 10㎕, it had higher ability to elicit glyceollin than in the water extract group significantly. But between the groups of 100㎕ and 1㎖ administration, it was no great difference except the extract group of fraction No. 2. But in the DMSO soluble(Et-OH extract) groups, there were also the ability of glyceollin elicitation when 10㎕ of extract was administrated even though it was lower than those of both water extract group sand Et-OH extract groups. From the above results it could be suggested that all of extracts had the ability to elicit glyceollins and these extracts could be helpful for plants to protect themselves from pathogenic

contaminations. It is necessary to do further studies to find out the molecule and how could cause the above glyceollin induction(Table 3).

Table 3. HPLC analysis of glyceollin elicitation

	glyceollin FN3(ng)	glyceollin FN2(ng)	glyceollin FN1(ng)
control	ND	ND	ND
10㎕	55.28± 7.35	33.32± 4.86	134.21±17.30
100㎕	34.03± 8.04	49.63±12.33	151.63±12.84
1㎖	ND	29.19± 7.58	186.03±22.15
Et-OH extract			
10㎕	38.65± 5.68	80.95± 8.45*	190.42±16.30*
100㎕	38.39± 8.24	81.51± 5.79*	183.11±12.75
1㎖	ND	87.96±10.41*	187.99±15.47
Et-OH extract (DMSO soluble)			
10㎕	66.58±11.32	ND	25.00±6.13**
100㎕	ND	ND	ND
1㎖	25.11± 9.28	ND	ND

Glyceollin FN1(fraction number 1) is the concentration of retention time of 5.78min, glyceollin FN2(fraction number 2) of 6.30min, glyceollin FN3(fraction number 3) of 9.52min. Data represent mean ±SD, * : $p< 0.05$, ** : $p < 0.01$(based on the value of water extract), n=3. ND means peak was not detected.
Water extract and Et-OH extract from 200g of sample were resuspended into 50㎖ of solvents respectively after vacuum concentration.

Effects on IAA-induced growth

For the pea segments grown on vermiculite bed for 7days at 25℃ in the dark without(Table 4) and with(Table 5) IAA, all water extracts had positive effects on the elongation of pea segments compared to the control value. In case of alcohol extracts and DMSO-soluble alcohol extracts, 100㎕ administration groups had positive effects also, but 1㎖ administration groups had not. And the positive effects of alcohol extracts and DMSO-soluble alcohol extracts were greater than those of water extracts. It seemed that there might be some synergistic effects between IAA and extracted samples on elongation of pea segments in all experimental groups positively determined. It is necessary to investigate more concerning what material in the extracts is involved and how it acts.

Table 4. Effects on pea stem elongation without IAA

	FW0	FWt	⊿FW	100x(C-T) /C(%)
water extract				
control(1㎖)	0.314	0.334	0.020	
100㎕	0.321	0.374	0.053	-165.0
1㎖	0.278	0.312	0.034	-70.0
Et-OH extract				
control(1㎖)	0.300	0.324	0.024	
100㎕	0.289	0.326	0.037	-54.2
1㎖	0.303	0.304	0.001	+95.5
Et-OH extract (DMSO soluble)				
control(1㎖)	0.270	0.302	0.032	
100㎕	0.307	0.350	0.043	-34.4
1㎖	0.268	0.272	0.004	+87.5

FW0 : fresh weight, FWt : fresh weight after incubation,
⊿FW = FWt - FW0 (increase in fresh weight),
100x(C-T)/C(%) = percent inhibition.

Table 5. Effects on pea stem elongation with IAA

	FW0	FWt	⊿FW	100x(C-T)/ C(%)
water extract				
control(1㎖)	0.278	0.303	0.025	
100㎕	0.306	0.388	0.082	-228.0
1㎖	0.311	0.357	0.046	-84.0
Et-OH extract				
control(1㎖)	0.302	0.313	0.011	
100㎕	0.305	0.388	0.083	-654.5
1㎖	0.314	0.317	0.003	+72.7
Et-OH extract (DMSO soluble)				
control(1㎖)	0.321	0.340	0.019	
100㎕	0.307	0.341	0.034	-78.9
1㎖	0.326	0.327	0.001	+94.7

FW0 : fresh weight, FWt : fresh weight after incubation,
⊿FW = FWt - FW0 (increase in fresh weight),
100x(C-T)/C(%) = percent inhibition.

요 약

사탕수수 찌꺼기를 여름느타리에 의해 발효한 후 부산물의 추출물로부터 생물학적 활성을 조사하기 위하여, 항종양성, 콩에 있어서의 glyceollin 유도성 및 완두에 있어서의 줄기 성장성을 관찰하였다. 알콜추출분획 및 DMSO분획물은 자실체 추출분획보다는 약했지만 매우 좋은 항종양성을 나타내었다. 콩의 자엽실험에서는 모든 추출물이 식물체로 하여금 병원성 감염을 방지할 수 있는 glyceollin 유도성을 갖고 있었다. 또한 IAA 유도 줄기성장 실험에서는 양성반응을 보인 모든 실험군에서 IAA와 추출물간의 유도 상승효과가 있는 것으로 나타났다.

중심단어 : *Pleurotus sajor-caju*, 사탕수수찌꺼기, 항종양성, glyceollin, 줄기성장성

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