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A Bioassay of Ginseng Extracts Based on Yeast Growth Determination

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ABSTRACT

For bioassay of the various extracts of ginseng, the growth determination method using *Saccharomyces cerevisiae* which was cultured with various doses of the extracts, was sdudied.

The water extract, powder and ethanol extract were more effective (about 45~110% increase) than saponins or its fractions (about 20~35% increase). The cold methanol residue showed a increase effect but it was not significant.

The bioassay curves for the water extract, ethanol extract, the butanol extracted saponins and the cold methanol-residue were made from the experimental data.

From these curves it is possible to find the relation between dose and effectiveness and the optimal doses of various ginseng extracts, and the amount of extract in a sample can be estimated.

The ranges of sample amount were 0.01% (100ppm)~0.32% (3200ppm) for the water extract, 0.025% (250ppm)~0.1% (1000ppm) for the ethanol extract, and 0.008% (80ppm)~0.016% (160 ppm) for the saponins. It was impossible to determine the range for the cold methanol-residue. The acceleration effects on the cell proliferation by a only 0.0008% (8ppm) of the diol- and triol-saponin were measurable in earlier period (24 hour treatment).

INTRODUCTION

Garrique[1] studied American ginseng (*Panax quinquefolium* L.) and succeeded in isolating a crude saponin which was named panaquilon. Several Japanese chemists, Ashahina[2] Kondo *et al.*[3-5] and Kotake[6] reported isolation of saponins or prosapogenin in ginseng, but they were not able to clarify the chemical structures. Shibata[7-9] demonstrated by thin layer chromatography (TLC) the occurrence of a number of saponins in ginseng which he named ginsenoside Rx (x= o, a, b_1, b_2, c, d, e, f, g_1, g_2, g_3, h_1, h_2) in sequence of spots on the TLC from bottom to top. Shibata group determined the chemical structures of some of the components of ginseng such as protopanaxadiol and protopanaxtriol and their derivatives[7-10]

The active components of ginseng influence not only animal cells but also fungi cells. Jung[11,12] reports that the rate of cell division of *Saccharomyces* treated with a water extract of ginseng was about twice than that of control group, and an optimal dose of the ginseng extract at 18℃ was more effective than a higher temperature (25℃) on the control group. Jung[13] showed

that cell division was increased by 53.3% on an optimal dose of ethanol extract. Kim *et al.* [14] noted about a 20% increase in cell division with a petroleum ether-ethanol extract under different culture conditions. Jung[15-17] and Kim *et al*[18]. have reported the other studies of ginseng effects on yeast also. Although these papers deal with the effects on yeast division of water extracts, ethanol extract, and petroleum ether-ethanol extract of ginseng and linoleic and stearic acid, these studies do not adequately explain what components are responsible for such effects.

Despite the many studies about ginseng, few papers about an assay method (Takahashi [19,20,21]; Woo *et al*,[22] Kim *et al.*,[23,24]) have been reported. And even these reports are not dealt with a bioassay for the various ginseng extracts. In order to determine and compare the effects of the various components or fractions of ginseng, it is necessary to establish a brief and effective method for the bioassay of ginseng. It was on the basis of above papers on *Saccharomyces* and the expected possibility of a bioassay using yeast that the author came to attempt a bioassay for ginseng extracts and fractions. For present study the above mentioned isolation methods of ginseng were used. The present paper provides a bioassay for ginseng extracts and fractions based on yeast growth determination.

MATERIALS AND METHODS

Four-year or six year old Korean white ginseng roots taken frpm Keumsan and Kangwha were used.

Saccharomyces cerevisiae were purely cultured from dry active yeast manufactured by Jeil-Universal Ltd. Co. (Lot No. Aug. 76 09005).

1. Various extractions of ginseng root

Powdered ginseng was extracted in a double boiler system with 10 times its volume of water for 4 hours and the water extract used for the yeast division tests.

The ginseng root was extracted with ethanol in a double boiler system for 10 days. The resulting ethanol extract (10.50%) was dried, then diluted with culture medium, and used for the yeast experiments.

Butanol extracted saponins were prepared by a modification of the procedures of Shibata *et al.*[8] and Woo *et al.*[25] The procedure is shown in Fig. 1. This saponins was used for the yeast experiments.

2. Fractionation of ginseng root

The ginseng powder was extracted according to the procedure, proposed by Shibata[26,10]. The powder was extracted with methanol in a double boiler system for 24 hrs, then concentrated in a vacuum evaporator. The concentrated extract was treated with 10 times its volume of methanol for 5 hours at 5℃. This was filtered and the residue (cold methanol-residue) used for the yeast experiment. The filtrate was concentrated and dried. Then, it was dissolved in ethylether. Shibata has shown that the ether precipitate contains the ginsenosides Ro, Ra to Rf and the ether supernatant contains the ginsenosides Rg_1, Rg_2, Rg_3. So these two groups were exa-

mined by TLC using solvent system A(n-butanol:glacial acetic acid:water=4:1:5, upper phase) and solvent system B(methanol:chloroform:water=35:65:10, lower phase).

The first group(1.0g) was applied to a silica gel column(50g, 1.7×50cm). The column was eluted with solvent system B and 5 ml fractions were collected. After every fraction tube were examined by TLC, 5 kinds of fractions were obtained and the 4th fractions were used in these experiments as diol-saponin.

The second group, the ether supernatant group, was treated as shown in the Shibata procedure[26] to obtain triol-saponin. The mixtures obtained from these two groups were diluted with glucose broth media and used for the yeast experiments.

3. Yeast culture and growth determination

The diluted preparations, agar media, broth media and glass apparatus were sterilized in an autoclave or oven.

Saccharomyees was cultured in glucose broth or glucose agar medium which added by a given dose of the extract or a fraction of ginseng as shown in Table 2~5. The experiment with the water-insoluble extract of ginseng was carried out using the procedure of Jung.[13] After the culture had proceeded for a given period, yeast was suspended by using a vibrator(Winsco, Seoul) and the number of cells were counted by Thoma hemocytometer, and on the saponins tests the total dried cells were weighed and on the cold methanol-residue tests spectrophotometrical absorbances of yeast suspension were detemined.

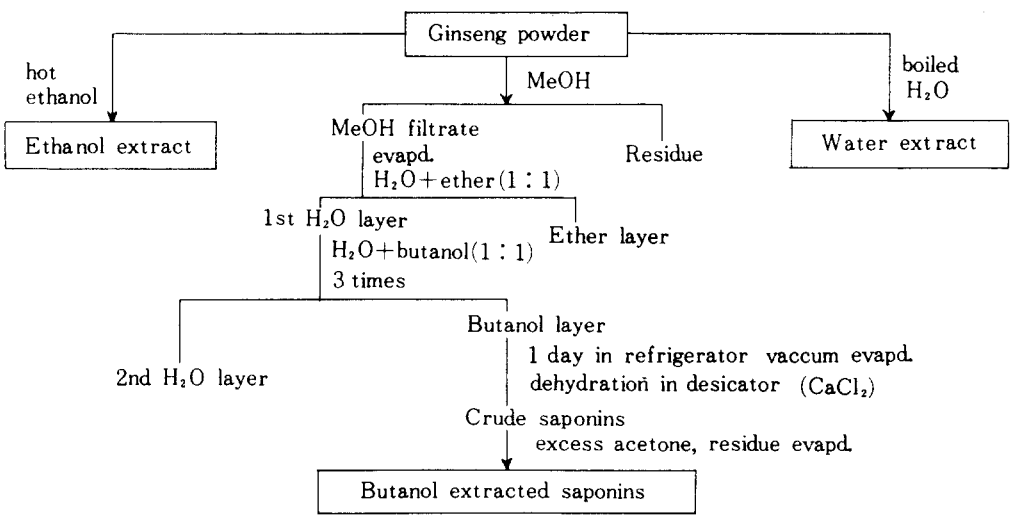

Fig. 1. Various extraction procedures for ginseng root.

RESULTS

1. Bioassay for the ginseng water extract

The rates of growth acceleration of yeast due to the water extract cultured for 24 and 48

hours at 28℃ are shown in Table 1. The medium contained 0.08% ginseng extract showed an optimal dose for the cell growth.

Ginseng from both the two areas showed similar effects. Bioassay curves for the water extract are plotted in Figure 2, 3, 4.

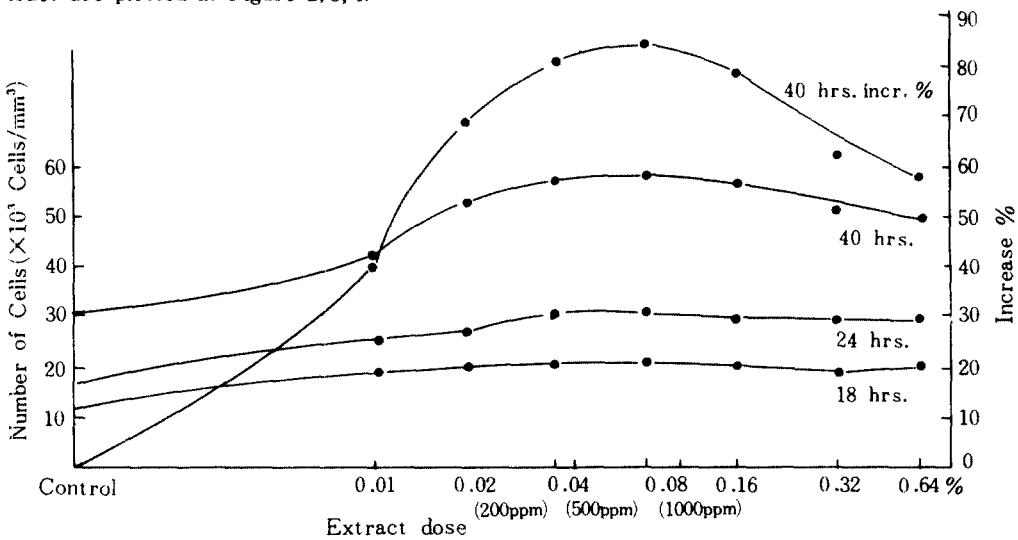

Fig. 2. Bioassay curves for the water extract of ginseng using *Saccharomyces cerevisiae** cultured at 25℃.

* Culture conditions:

1) Culture medium: Moyer and Coghill's (pH :6.0). 5 ml.
2) Culture apparatus: 15 ml test tube with cotton plug.
3) Initial cell density: 10 cells/mm³
4) Ginseng extract dose: Weight % in the broth medium based on the weight of dry ginseng root.

* Count: Mean of 8 counts of 2 tubes by Thoma hemocytometer.

Table 1. Proliferation of *Saccharomyces** treated with water extracts of Korean ginseng at 28℃ (×10³ cells/mm³)

Extract dose \ Hrs cultured		0	24 hrs	48 hrs	Rates of increase (%) 24 hrs	48 hrs
K_1**	Control	10^2	10.25±1.17	23.00±2.87	0	0
	0.04%	〃	15.50±1.41 †	31.00±2.99 †	51.22	34.78
	0.08%	〃	18.25±2.19	40.17±2.84	78.05	74.65
	0.16%	〃	14.75±1.46 †	38.33±2.04	43.90	38.33
K_2**	Control	〃	10.50±1.26	23.38±0.41	0	0
	0.04%	〃	14.00±0.82 †	31.13±1.49	33.33	33.15
	0.08%	〃	17.50±1.55	41.13±1.78	66.67	75.92
	0.16%	〃	16.13±0.95	35.00±2.35	53.62	49.70

$P < 0.01$, † $P < 0.05$ to control.

* *Saccharomyces* were cultured on the glucose broth medium in air. ** Area of production.

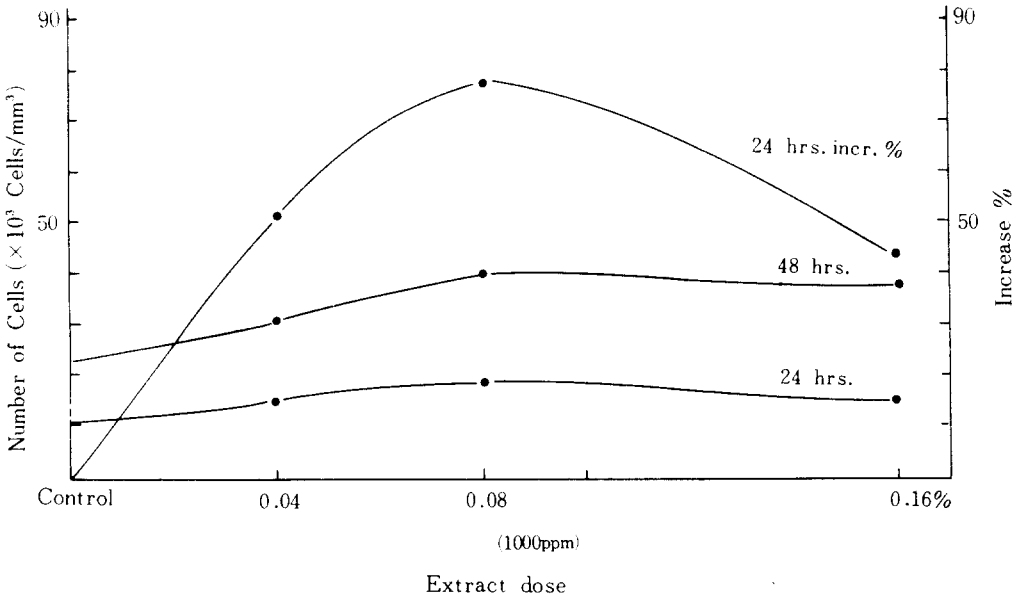

Fig. 3. Bioassay curves for the water extract of ginseng (K_1 area) using *Saccharomyces cerevisiae** cultured at 28℃.

* Culture conditions: 1) Culture medium : Glucose broth. (pH 6.0).
Culture apparatus : 25 ml flask with cotton plug.
Initial cell density and extract dose are the same as in Fig. 2.

* Count : Mean of 8 counts of 2 tubes by Thoma hemocytometer after shaking by Winsco Vibrator.

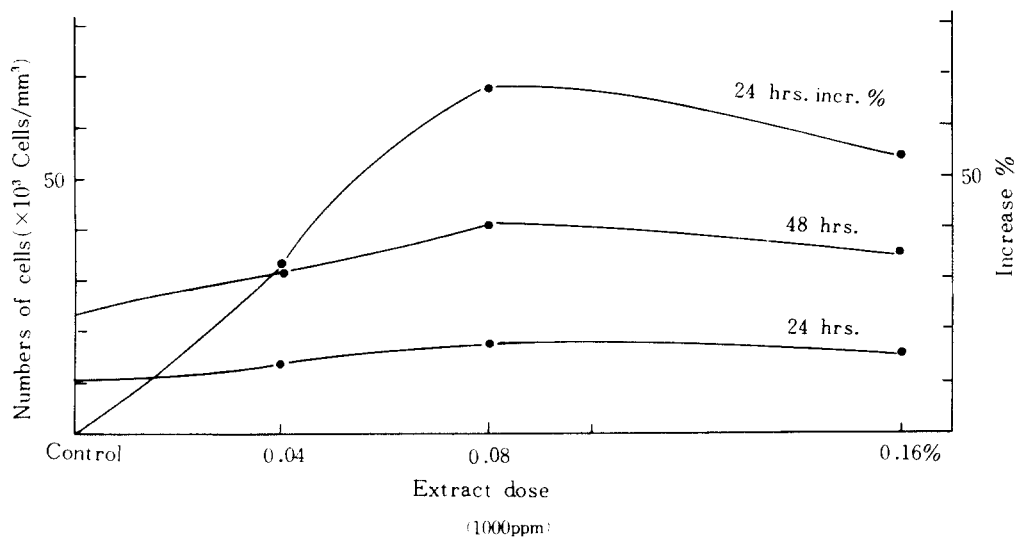

Fig. 4. Bioassay curves for the water extract of ginseng (K_2 area) using *Saccharomyces cerevisiae** cultured at 28℃.

* Culture conditions and count method are the same as in Fig. 3.

2. Bioassay for butanol extracted saponins

The rates of growth acceleration of yeast due to butanol extracted saponins on 24 and 48 hours cultures at 28℃ are shown in Table 2. In this case the optimal dose of saponins was a 0.008% saponins medium which is equivalent to 1/10 of the water extract. The rates of increase for the saponins were much less than those of the ginseng water extract. The number of cells and the dry cell weight were increased similarly. Bioassay curves for the saponins are shown in Fig. 5.

Table. 2. Proliferation of *Saccgromyces** treated with butanol extracted saponins of Korean ginseng at 28℃.

	Count	Number of cells($\times 10^3$cells/mm^3)			Dry wt. (mg)
Saponins dose \ Hrs cultured		0	22	48	48
	Control	10^{-2}	6.38±0.78	22.25±2.54	4.7
	0.004%	〃	7.63±0.63	25.13±0.81	5.5
	0.008%	〃	8.00±1.12	29.88±1.34 †	6.2
	0.016%	〃	8.13±0.61 †	26.38±0.61 †	5.7
Rate of	0.005%	—	19.60	12.94	17.02
increase	0.008%	—	25.49	34.29	31.92
(%)	0.016%	—	27.43	18.54	21.28

* See footnote to Table 1. † $P < 0.05$ to control.

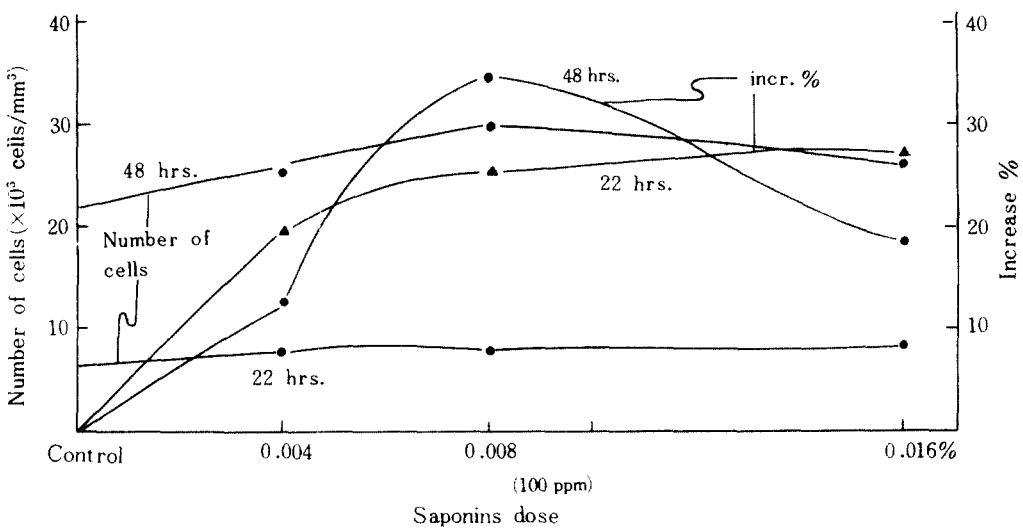

Fig. 5. Bioassay curves for butanol extracted saponins of Korean ginseng using *Saccharomyces cerevisiae** cultured at 28℃.

* Culture conditions 1), 2), 3) and count method are the same as in Fig. 3. Dose of saponins : Dry weight % of saponins itself.

3. Bioassay for the ginseng cold methanol-residue and ethanol extract

On slant agar tubes containing 0.04% and 0.08% cold methanol-residue the yeast growth was increased, but it was not significant. Table 3 shows the number of cells and the absorbance of yeast suspension, and the rates of increase were similar. Bioassay curves for cold methanol-residue and ethanol extract are presented in Fig. 6.

Table 3. Proliferation of *Saccharomyces** treated with cold methanol-residue of ginseng (×3×10 cells/mm³/1 colony, suspended in 10 ml of water).

Treatment	Number of Cells	Rate of increase(%)	Absorbance of cell** suspension at 560mμ	Rates of increase(%)
Control	7.625±0.61	0	0.76	0
0.04%	8.111±0.72	6.37	0.82	7.90
0.08%	9.222±0.83	26.00	0.95	25.00
0.16%	7.625±0.89	0	0.75	-1.32
0.32%	7.556±0.63	-0.91	0.72	-5.26

The means are not significant to control.

**Saccharomyces* were cultured on glucose agar medium for 42 hours 28℃ in air.

***Comparison data of *Saccharomyces* suspension by spectrophotometry.

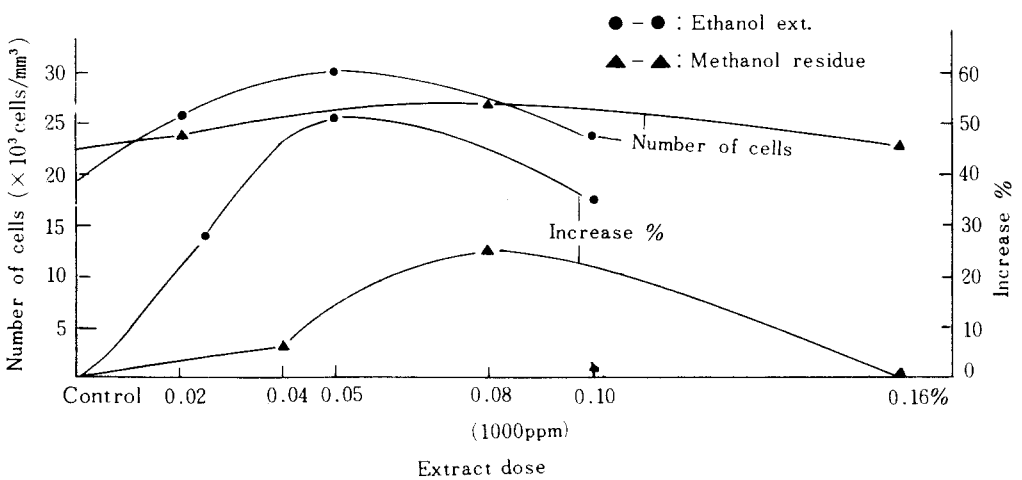

Fig. 6. Bioassay curves for ginseng ethanol extract and cold metanol-residue using *Saccharomyces cerevisiae** cultured at 28℃.

* Culture conditions : 1) Culture medium : Glucose agar medium. (pH 6.1) 2) Culture apparatus : Petri dish or 50 ml. test tube. 3) Colonies were cultured for 40-42 hours after streaking. 4) Extract dose : Dry weight % of extract itself.

* Count : Mean of 10 colonies. Cell number unit : ×3×10² cells/mm³/1 colony, suspended in 10 ml of water.

4. Optimal extract or fraction doses

For bioassay by the yeast growth determination it is important to ascertain the optimal doses. The optimal doses for several extracts and fractions obtained from various experiments are shown in Table 4. The water extract, powder and ethanol extract were effective and the rates of growth acceleration of yeast were 45~110%. Butanol extracted saponins, diol- and triol-saponin were less effective than the other extracts. Even 0.0008% diol- and triol-saponin promoted the proliferation about 20~35% at 24 hours.

Table 4. Optimal doses for several extracts or fractions of ginseng and their rates of increase on *Saccharomyces* culture.

(Initial cell density : $10/mm^3$)

Treatment	Optimal dose (wt. % in medium)	Max. rate of increase (%)	incubation temp. (℃) and time (hrs)		Medium
Powder*	0.10	145.98	25	40	Moyer and Coghill
〃 *	〃	211.64	25	56	〃
Water extract*	0.08	206.83	25	52	〃
〃 (K_1)	〃	174.65	28	48	Glucose broth
〃 (K_2)	〃	175.92	〃	〃	〃
Ethanol extract**	0.05	153.30	28	40	Glucose agar
Powder**	0.10	144.23	〃	〃	〃
Cold MeOH-residue	0.08	126.00	28	42	〃
Butanol extracted saponins	0.008	134.29	28	48	Glucose broth
Diol-saponin	0.0008	135.91	28	24	〃
〃	〃	118.36	〃	48	〃
Triol-saponin	〃	121.74	28	24	〃
〃	〃	107.78	〃	48	〃

* Results of previous paper (Jung, 1969a)[12] ** (Jung, 1969c)[13]

DISCUSSION

In the selection of a appropriate living material for the bioassay of various ginseng extracts the following conditions would be considered. (1) One kind of material should be used for the determination of effects of various ginseng extracts or fractions. (2) The effects of various concentrations of the extracts should be shown continuous tendency. (3) The material must exhibit a definite effect for each extracts. (4) The material should be easily cultured, and the possibility of other changes arising due to the various environmental and internal factors should be minimizable. (5) The method for determining the effects of several experimental groups should be brief and valid. (6) If the material is cellular level, the cell material should have similar functions in metabolism with other multicellular organism.

The materials meeting the first three conditions could include experimental animals such as mice, rats, or rabbits etc. However the latter three conditions could be met with an unicellular organism. Though yeast is an unicellular organism, which lives in both aerobic and anaerobic conditions as well as the cells of multicellular organism. A brief culture and growth determination method can be easily applied to yeast. Therefore yeast will be useful as a good material for the bioassay of ginseng extracts. The present study of ginseng on yeast has confirmed the several studies carried out by Jung[12,13,15-17] and Kim *et al.*[14,18]

The determining method of several effects for bioassay should be valid. The present method for estimating the mean of 8 counts in 2 experimental tubes by Thoma hemocytometer was significant. Before yeast cells could be counted Kim *et al.*[14] used an ultrasonificator to make a homogeneous suspension of yeast. The errors of data were ±10%. As shown in the results of the present experiment using a viberator most of the errors were in a range of 5%. By comparing the cell counts, the dry cell weight and spectrophotometrical absorbance for yeast suspension, it was showed that the cell counts were useful for the water extract or powder bioassay. The latter two methods are useful for the saponins or its fractions also.

The optimal doses for the various extracts were different for the yeast growth. The water extract, powder and ethanol extract were more effective than the saponins or its fractions, so it can be said that the effect of ginseng on yeast growth depends not only upon the saponins or its fractions but also on other components of ginseng such as fatty acids[13] and unknown components. Therefore bioassay curves for ginseng would be necessary for the various extracts and components of ginseng(Fig. 2-6).

The bioassay curves may be used for the determination of the amount of extract or fraction of ginseng contained in a sample. The optimal dose of the extract or fraction in a sample must be determined first, and then using a less or more diluted sample than the optimal dose, make a curve which can be compared with the bioassay curve to find the amount of ginseng extract or fraction. From the data of Table 4 it is possible to assume the optimal dose of various extracts or fractions in samples.

For use of present bioassay, the experimental conditions such as culture method, media, temperature and period, and initial cell density should be maintained constantly.

효모성장측정을 이용한 인삼추출물의 생물학적 검정

鄭 魯 八

요 약

인삼의 여러가지 추출물의 효과를 검정하는 생물학적 방법의 하나로 여러 농도의 추출물을 배지에 첨가하여 배양한 *Saccharomyces cerevisiae*의 증식을 측정하는 효모 이용법을 연구하였다.

인삼의 물추출물, 분말, 에탄올추출물의 최적량에서 최고 증식촉진 효과(약45~110%)

는 인삼사포닌이나 그 분획물의 효과(약20~35%)보다 더 효과적이었다. 냉메탄올 - 잔류물은 약간의 촉진효과(26%)를 보였으나 통계학적으로 유의성이 없었다.

증식촉진 효과의 자료에 의하여 물추출물, 에탄올 추출물, 사포닌, 냉메탄올 - 잔류물의 효과 검정곡선을 작성하였다. 이 곡선을 이용하면 투여량과 효과성과의 관계 및 추출물의 촉진 최적량을 알 수 있다. 또 여러가지 피검정추출물(피검물)을 여러가지 비율로 희석하여 효모 배양을 하였을 때 작성되는 피검물곡선을 표준검정곡선에 대조하면 피검물양을 생물학적으로 정할 수 있다.

피검물의 측정가능 범위는 물추출물이 0.01% (100ppm) - 0.32% (3200ppm). 에탄올추출물이 0.025% (250ppm) - 0.1% (1000ppm), 사포닌이 0.008% (80ppm) - 0.016% (160ppm)이었다. 그 최적량은 각각 0.08%, 0.05%, 0.008%이었다. 냉메탄올 - 잔류물은 최적량이 0.08% (26%촉진) 이었으나 유의성이 없었기 때문에 측정가능 범위를 정하지 못하였다. 디올계 사포닌과 트리올계 사포닌은 0.0008% (8 ppm)에서 증식 촉진 효과를 나타내었는데 초기인 24시간에 더 효과적이었다.

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