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Effect of Korea Ginseng Root on Detoxification of Heavy Metal, Mercury by *Fusarium oxysporum*

Young Ho Kim§, Eun Kyung Park and Kyu Jin Park
Korea Ginseng & Tobacco Research Institute, Taejeon 305-345
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Abstract□Extracts of *Panax ginseng* root significantly induced tolerance of *Fusarium oxysporum* to heavy metal, mecury, as the fungal mycelial growth was less inhibited by mercury chloride on potato dextrose medium(PDA) amended with ginseng root than on the PDA with no ginseng amendment. The most favorable concentration of ginseng root powder in detoxification of mercury chloride was 1%. The induced tolerance of *F. oxysporum* to mercury chloride appeared to be rather due to absorption of ginseng components, and was not related to stimulation of mycelial growth of the fungus *per se* by ginseng treatment. Ginseng components responsible for inducing tolerance of the fungus to mercury were involved in the water fraction of the ginseng root extract, althought the water fraction had no effect on enhancement of the mycelial growth on the medium without mercury chloride. The hexane fraction of ginseng root extract, by which the mycelial growth was stimulated, was not related to the inducement of the tolerance to mercury chloride. However, more tolerance to mercury chloride was noted in PDA with both the water and hexane fractions combined than with either of the two fractions. Six-year-old ginseng roots were more effective in detoxification of mercury chloride than 4-year-old ginsng roots, and American ginseng (*P. quinquifolium*) had no or little effect on inducing tolerance of the fungus to mercury chloride. This method may be used to screen other natural materials for test in the detoxification of mercury chloride.

Key words□*Panax ginseng, Fusarium oxysporum,* induced tolerance, mercury chloride

Introduction

The efficacy of ginseng has often been scientifically studied by using microorganisms owing to simplicity and rapididity in determining active components of ginseng and in evaluating biochemical and physiological activities of the microorganisms affected by ginseng extracts.[1-7] So far, most studies have been limited to assay of the relations in ginseng and microorganisms *per se.*

Little study has been reported on the protective effect of ginseng to hazardous materials by using microorganisms, although the role of *Panax ginseng* in detoxification of xenobiotics[8] and in prevention of human or animal cells and tissues from toxicity by heavy metals such as cromium[9,10] has been well documented. Prior to clinical and *in vivo* assays of the efficacy of ginseng, it is required that a rapid screening method should be developed so as to select possible acitive components related to the efficacy.

Therefore, this study was designed to develop a screening method of investigation of effect of ginseng roots on the detoxification of mercury by *Fusarium oxysporum.*

Materials and Methods

1. Preparation of the fungal inoculum and culture of the fungus

Fusarium oxysporum(IFU 9761) was cultured on potato dextrose agar medium(PDA) (Difco) for 10 days at 25~27℃, and spores of the fungus were collected in sterilized distilled water by pouring

§ To whom correspondence should be addressed

about 30 ml of the water into the cultural plate followed by smearing the surface of the cultural medium to separate spores from the medium. For culture of the fungus, 0.2 ml of the spore suspension was placed on PDA with or without amendment by ginseng and spread evenly on each agar medium(8 ml) in a petri dish(9 cm, ϕ), which was placed in a incubator at 25~27 ℃ in the darkness. The same conditions mentioned above were used for the preparation of the fungal inoculum and the fungal culture throughout this experiment unless otherwise mentioned.

2. Preparation of ginseng medium

Six-year-old dried roots of *Panax ginseng* C.A. Meyer, 4- or 6-year-old Korean red and white ginseng roots, and dried 4-year American ginseng roots(excluding fine roots) were ground to powder. For amendment of ginseng root in culture medium, 4g of ginseng powder was suspended in 100 ml distilled water and autoclaved for 20 min. The ginseng suspension was filtered with Watman No. 2 filter paper, and the filtered solution was made to 100 ml by adding distilled water, diluted to proper concentrations by mixing the ginseng solution with distilled water. The same amount of PDA powder as PDA medium used for the control was added into the diluted solutions prior to autoclaving, so that the concentration of ginseng root extract was made equivalent to the amount of root powder included in the cultural media.

3. Separation and amendment of ginseng extract in culture medium

A hundred grams of dried 6-year-old ginseng root powder were exracted in 2000 ml of 80% ethanol, and the extract solution was evaporated to dryness. The ethanol extract of ginseng roots was separated in a separation funnel by using 100 ml n-hexane and 100 ml distilled water two times. The water layer was futher separated in a series of chloroform and butanol layers, using equal volumes of chloroform and n-butanol to that of the water layer, respectively. Each solution separated was evaporated to dryness, and 100% ethanol was added to each extract to make 100 ml solution. The ethanol solutions of root extracts were added into distilled water to make the concentrations equivalent to the percentages of ginseng root. PDA powder was added into the solutions and autoclaved.

4. Inhibition of fungal growth by mercury chloride

Pure mercury chloride was dissolved in sterilized distilled water. A paper disc(8 mm ϕ) was soaked with 0.06 ml of the mercury chloride solution. The paper disc soaked with mercury chloride solution was placed in the center of the plate containing 8 ml PDA or ginseng-amended PDA medium, following inoculation of fungal spores as mentioned above. The diameters of inhibition zones formed around the paper discs were measured.

5. Mycelial growth of the fungus

Mycelial discs(7 mm, ϕ) grown on ginseng-amended PDA or pure PDA medium for 7 to 10 days were placed on the center of petri dish containing 8 ml PDA medium with or without ginseng amendment. The colony diameters of the fungus were measured from 2 days after inoculation.

Results

1. Effect of ginseng amendment on growth of the fungus in different concentrations of mercury chloride

One day after inoculation of the fungus, an inhibition zone appeared around the paper disc soaked with mercury chloride. After 2 days, the inhibition zones were more clearly shown with no noticeable mycelial growth into the center of the plate. The radii of inhibition zone were proportional to the concentration of mercury chloride, regardless of ginseng amandment; however, larger inhibition zones were always made on PDA than on the ginseng amended agar medium(GAA) at the same concentration of the heavy metal(Table 1). After 6 days, sizes of inhibition zone differed more greatly between the PDA and GAA media(Table 1), indicating that the fungal mycelium readily grew on the medium treated with ginseng root extract.

2. Optimum concentration of ginseng root extract

Inhibition zones appeared 1 and 3 days after inoculation were not significantly different among the control, 0.1, 0.2 and 0.5% of ginseng root extract,

Table 1. The influence of mercury chloride on the growth of *Fusarium oxysporum* on potato(PDA) and ginseng-amended PDA(GAA)[1]

Concentration[2] of mercury chloride	Inhibition radius(mm) after[3]					
	2days			6days		
	PDA	GAA	P	PDA	GAA	P
Control	0.0	0.0		0.0	0.0	
0.1%	19.6± 0.4	17.3± 0.5	***[4]	10.4± 1.0	5.8± 1.3	***
0.2%	22.0± 0.5	18.8± 0.9	***	12.8± 1.9	6.5± 0.8	***
0.5%	26.9± 2	23.8± 0.1	***	22.1± 2.4	11.1± 2.4	***
1.0%	30.7± 0.6	25.9± 2.4	**	30.0± 0.9	18.9± 1.8	***

[1]Powder of 6-year old ginseng roots was autoclaved in distilled water and filtered. The filtered ginseng solution was incorporated into the PDA medium, making 1.0% ginseng-amended medium.
[2]A paper disc(8 mm in diameter) was soaked with 0.06 m*l* of mercury chloride, and placed in the center of the agar medium, follwing inoculation of the fungal spores on the medium.
[3]Numbers are averages± standard deviations of 4 replicates.
[4]Significant at p=0.05(*), p=0.01(**), and p=0.001(***).

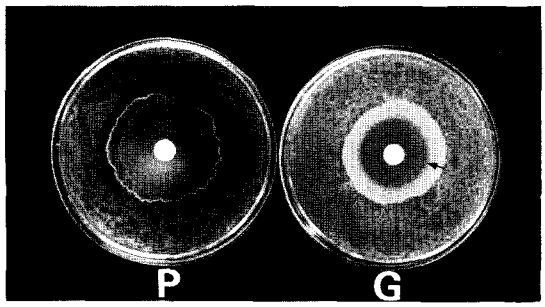

Fig. 1. Inhibition zones formed around paper discs soaked with 0.06 m*l* of 0.5% mercury chloride 6 days after inoculation with *Fusarium oxysporum* on PDA(P) and PDA amended with 1% of ginseng root extract(G). Note the smaller inhibition zone on(G) and the mycelium grown into the center of the plate(arrow), but no mycelial growth on(P).

while significant difference was noted after 6 days of fungal growth between GAA and PDA medium (Table 2). In 0.1% and 2.0%, the radii of inhibition zone were significantly different from the control at 1 and 3 days after inoculation, and more greatly at 6 days after inoculation, of which 1.0% of the ginseng root extract formed the smaller inhibition zones than 2.0%. This indicates that the optimum concentration may be 1.0% to test antidotal efficacy of ginseng root extract against mercury chloride.

3. Mechanism of tolerance to mercury chloride induced by ginseng root

Table 2. Effect of ginseng amendment on the growth of *Fusarium oxysporum* influenced by mercury chloride[1]

Concentration[2] of ginseng	Inhibition radius(mm) after[3]		
	1day	3days	7days
0.0%	18.5± 0.8b[4]	18.5± 0.8c	16.6± 0.9b
0.1%	18.7± 0.1b	18.0± 0.5c	15.1± 0.8b
0.2%	18.8± 0.4b	18.5± 0.7c	15.6± 0.7b
0.5%	18.4± 0.7b	17.7± 1.3c	14.4± 1.9b
1.0%	15.7± 0.5a	10.7± 1.3a	7.2± 1.0a
2.0%	17.8± 1.0b	12.1± 2.6b	9.1± 1.3a

[1]A paper disc(8 mm in diameter) was soaked with 0.06 m*l* of 0.5% mercury chloride, and placed in the center of the agar medium, follwing inoculation of the fungal spores on the medium.
[2]Powder of 6-year old ginseng roots was autoclaved in distilled water and filtered. The filtered ginseng solution was incoporated into the PDA medium with the appropriate percentages equivalent to the total weight in the medium.
[3]Number are averages± standard deviations of 4 replicates.
[4]The same letters on each column are not different at p=0.05 by pooled standard deviation.

Spores of *F. oxysporum* obtained from PDA or GAA cultures were subjected to the antidotal test to mercury chloride. At 2 days after inoculation, the inhibition zones of the inoulum from GAA on PDA or GAA, and that from PDA on GAA were

Table 3. Influence of ginseng amendment on the induced tolerance of *Fusarium oxysporum* from PDA and ginseng-amended PDA(GAA) cultures to mercury chloride[1)]

Cultural medium	Source[2)] medium	Inhibition radius(mm) after[3)]	
		2 days	6 days
PDA	PDA	18.5± 0.7b[4)]	15.4± 1.6b
	GAA	16.9± 1.3a	14.3± 3.4b
GAA	PDA	17.2± 0.7a	9.8± 0.8a
	GAA	17.0± 1.0a	9.6± 1.0a

[1)]Powder of 6-year old ginseng roots was autoclaved in distilled water and filtered. The filtered ginseng solution was incoporated into the PDA medium, of which the percentage of ginseng extract was equivalent to 1% of ginseng root in the medium. A paper disc(8 mm in diameter) was soaked with 0.06 m*l* of 0.5% mercury chloride, and placed in the center of the agar medium, follwing inoculation of the fungal spores on the medium

[2)]The fungus was cultured on PDA or GAA medium for 10 days at 27 ℃

[3)]Numbers are averages± standard deviations of 4 replicates

[4)]The same letters on each column are not different at p=0.05 by pooled standard deviation

signficantly smaller than that from PDA on PDA (Table 3). However, significant mycelial growth into the paper disc treated with mercury chloride was noted only on GAA, regardless of the source medium that the inoculum had been originated.

Fugal mycelium discs from the both PDA and GAA were placed in the center of the PDA and GAA with no treatment of mercury chloride, and growth of the mycelium was measured. In this test, the mycelial growth was not significantly different among the treatments(Fig. 2).

4. Ginseng fractions responsible for inducement of tolerance to mecury cholide

The inhibition zone of the fungus was the smallest on the medium amended with water fraction of ginseng root extract 1 day after inoculation. At 8 days after inoculation, the significant difference in the size of inhibition zones from the control was noted only on the medium amended with the water fraction of ginseng extract, while hexane, butanol and chloroform fractions showed no significant effects on the detoxification of mercury chloride by

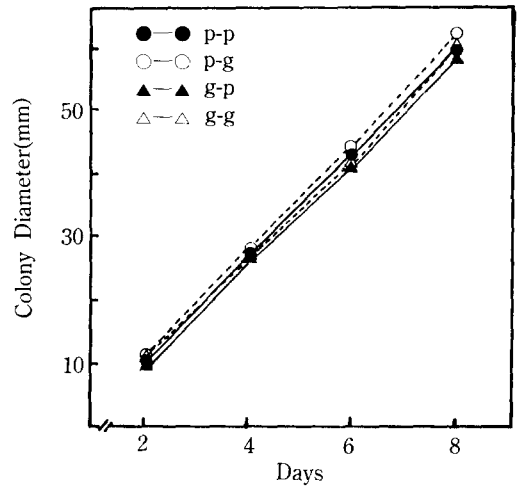

Fig. 2. Mycelial growth of *Fusarum oxysporum*, of which the inocula were originated from PDA (p-) and ginseng-amended PDA(g-) cultures, on PDA(-p) and ginseng-amended PDA(-g).

the fungus.

5. Fungal growth in the medium with ginseng fractions

In the media without mercury chloride, no significant difference was shown in the mycelial growth among the control, water and chloroform fractions (Fig. 3). In the hexane fraction, the mycelial growth was significantly enhanced, while in the butanol fraction it was inhibited, compared with the mycelial growth in the control.

6. Mixture effect of the water and hexane fraction of ginseng

Water and hexane fractions, which induced tolerance to mercury and stimulated fungal growth, respectively, were incorperated in PDA medium singly or in combination. In this test, water fraction with not less than 2% significantly induced the tolerance of the fungus aganist mercury chloride, while hexane fraction had no significant effect in the inducement of tolerance(Table 5). Mixture of water and hexane fractions, however, had more prominent effect in protecting the influence of mercury chloride than the water fraction only at the same levels of concentration.

7. Comparison of induced toleranced by different species and ages of ginseng

One day after inoculation, radii of inhibition zone

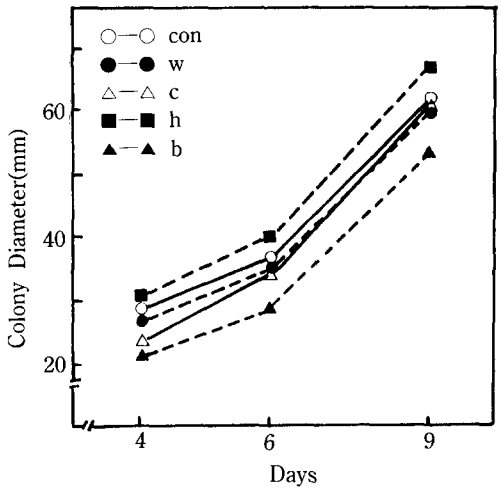

Fig. 3. Mycelial growth of *Fusarium oxysporum* on PDA amended with each fraction of ginseng extract (con: control, w: water fraction, c: chloroform fraction, h: hexane fraction, and b: butanol fraction).

Table 4. Effect of ginseng amendment on the mycelial growth of *Fusarium oxysporum* against mercury chloride on PDA medium[1)]

Ginseng[2)] fraction	Inhibition radius(mm) after[3)]		
	2 days	4 days	8 days
Control	19.3± 0.8b[4)]	19.3± 0.8b	18.3± 1.0b
Water fraction	18.0± 0.8a	17.0± 0.7a	12.7± 1.2a
Butanol fraction	20.7± 0.9bc	20.7± 0.9c	19.6± 0.6b
Chloroform fraction	19.9± 0.9bc	19.9± 0.9bc	18.9± 0.9b
Hexane fraction	21.2± 0.4c	20.6± 0.4b	18.2± 0.7b

[1)]A paper disc(8 mm in diameter) was soaked with 0.06 m*l* of 0.5% mercury chloride, and placed in the center of the agar medium, follwing inoculation of the fungal spores on the medium

[2)]Each solution fractionated by the solvent was incoporated into PDA. The concentration of each fractioned solution was made to be equivalent to 2% of the root weight in the medium

[3)]Numbers are averages± standard deviations of 4 replicates

[4)]The same letters on each column are not different at p=0.05 by pooled standard deviation

were significantly smaller in Korean ginseng and American ginseng from U.S.A. than in the control. However, at 5 days after inoculation, American ginseng had no significant difference in the size of

Table 5. Effect of water(WF) hexane(HF) fractions by ginseng on the mycelial growth of *Fusarium oxysporum* against mercury chloride on PDA medium[1)]

Ginseng[2)] fraction	Inhibition radius(mm) after[3)]		
	2 days	4 days	6 days
Control	21.5± 0.6b[4)]	21.5± 0.6c	8.3± 0.5c
WF 1%	21.6± 1.0b	21.6± 1.0c	20.1± 1.4c
WF 2%	20.3± 0.5ab	18.8± 0.5b	16.8± 0.3b
WF 4%	19.4± 0.9a	16.7± 1.2a	12.9± 1.2a
HF 1%	21.6± 0.3b	21.6± 0.3c	20.1± 1.0c
HF 2%	20.1± 1.0ab	20.1± 1.0bc	20.0± 0.5c
HF 4%	21.4± 0.8b	21.4± 0.8c	20.3± 0.6c
WF 1%+HF 1%	20.7± 0.4ab	20.1± 0.7bc	17.7± 0.4b
WF 2%+HF 2%	19.8± 0.5a	19.8± 0.5bc	14.0± 0.5a

[1)]A paper disc(8 mm in diameter) was soaked with 0.06 m*l* of 0.5% mercury chloride, and placed in the center of the agar medium, follwing inoculation of the fungal spores on the medium

[2)]Each solution fractionated by the solvent was incoporated into PDA. The concentration of each fractioned solution was made to be equivalent to the root weight in the medium

[3)]Numbers are averages± standard deviations of 4 replicates

[4)]The same letters on each column are not different at p=0.05 by pooled standard deviation

inhibition zone from the control. The six-year-old Korean red ginseng had the smallest inhibition zone, and 6-year-old Korean ginseng had more detoxification effect of mercury chloride than 4-year-old Korean ginseng. Red ginseng had slghtly more, but not significantly different, antidotal effect than white ginseng in general.

Discussion

In this experiment, Korean ginseng root increased the detoxification capacity of mercury by *F. oxysporum* since in the PDA amended with ginseng root extract the mycelial growth of the fungus was less inhibited by mercury chloride than the PDA without ginseng treatment. Detoxification material was present in water fraction of the ginseng extract, and was not related to stimulation of the mycelial growth in itself in inducing tolerance to the heavy metal. Because the fungal inoculum originated from

Table 6. Comparision of detoxification of mercury chloride in different species and ages of ginseng[1)]

Species[2)]	Age	Type	Inhibition radius(mm) after[3)]	
			1 day	5 days
Control			20.8± 0.4b	17.2± 0.7b
Korean ginseng	6-year	Red ginseng	19.5± 0.7a	13.7± 1.0a
	6-year	White ginseng	19.5± 0.7a	14.7± 0.8a
	4-year	Red ginseng	20.2± 0.7ab	15.2± 0.9ab
	4-year	White ginseng	19.6± 0.3a	15.6± 0.3bc
American ginseng(Canada)	4-year	White ginseng	21.1± 0.6b	17.9± 0.7d
American ginseng(USA)	4-year	White ginseng	20.3± 0.5ab	16.6± 0.3cd

[1)] A paper disc(8 mm in diameter) was soaked with 0.06 m*l* of 0.5% mercury chloride, and placed in the center of the agar medium, follwing inoculation of the fungal spores on the medium
[2)] Powder of ginseng roots was autoclaved in distilled water and filtered. The filtered ginseng solution was incoporated into the PDA medium, in which the percentage of ginseng was equivalent to 1% of root weight
[3)] Numbers are averages± standard deviations of 4 replicates
[4)] The same letters on each column are not different at p=0.05 by pooled standard deviation

the ginseng-amended medium initially formed smaller inhibition zone by mercury chloride than that from PDA without ginseng treatment, the fungus might have absorbed detoxification material, probably present in the water fraction of the ginseng extract, that may influence the detoxification of mercury chloride by the fungus.

The optimal concentration of ginseng extract appeared to be 1.0%, while that of water fraction of ginseng root extract might be 4% or more. This indicates that the less effectiveness in detoxification of mercury at the higher concentration of total ginseng extract may be caused by other ginseng components. Hexane fraction of the ginseng extract might enhance the detoxification of mercury in the medium treated with the water fraction of the ginseng extract by stimulating the mycelial growth.

In soil, toxic mercury compounds are transformed to less harmful ones by forming volatile elemental mercury and dimethyl mercury[11,12)] or reduction of Hg^{2+} to Hg^{0} which is related to enzymatic reactions by microorganisms.[13)] Or the toxic mercuric compounds are immorbilized in soli by adsorption to negatively charged materials such as clay particles.[14)] Not all the natural materials had remarkable effect on the detoxification of mercury as in ginseng (unpublished). Also in this study different detoxification effects were noted in different species and ages of ginseng, suggesting that simple adsorption of the mercury compound to organic materials may not be related to the mechanism of detoxification of mercury. In this experiment, the material responsible for the detoxification of mercury and the detoxification reaction were not examined; however, some ginseng components soluble in water or materials of which the production was stimulated by the ginseng components may have reduced the harmful effect of mercury chloride.

Kim *et al.*[15)] demonstrated the effect of ginseng saponin on the antimicrobial activities of some antibiotics. They tested the effect in broth cultures of bacteria treated with both antibiotics and ginseng saponin. In our study, the detoxification effect was clearly visible without help of any sophiscated apparatus and could be quantitatively measured by mycelial growth of the fungus on the solid media. Therefore, the screening method is simple, and may be used for examining of natural materials on the detoxification of mercury and identification of the active components.

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요 약

高麗人蔘의 뿌리추출물을 첨가한 감자한천 배지에서 승홍에 의한 *Fusarium oxysporum* 生長의 저지효과가 낮고, 수은의 영향에도 불구하고 菌絲生育이 양호하여 고려인삼이 重金屬 水銀의 解毒作用이 있는 것으로 나타났다. 승홍의 해독작용에 가장 적합한 인삼의 농도는 1.0%였다. *F. oxysporum*의 승홍에 대한 耐性誘發에 있어서 인삼의 영향은 곰팡이에 의한 인삼성분의 흡수와 관련이 있는 것으로 나타났으며, 균사생장을 촉진하는 인삼의 성분과는 관련이 없었다. 수은의 解毒作用과 관련이 있는 인삼의 성분은 인삼의 추출물등 물층에 있었으며, 인삼추출물의 Hexane 층은 균사생장을 촉진하였지만 수은의 解毒作用에는 관여하지 않는 것으로 나타났다. 그러나 물층과 Hexane 층의 물질을 동시에 처리하였을 때에는 승홍의 해독작용 효과가 상승하였다. 인삼의 종류별 승홍의 解毒作用에 있어서는 6년근이 4년근보다 그 효과가 컸으며, 미국인삼은 이러한 重金屬 解毒能力이 거의 없는 것으로 나타났다. 이 방법은 천연물에 의한 중금속 수은의 解毒作用에 대한 효과를 조사하는데 유용하게 사용될 수 있을 것으로 생각된다.

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