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Antifungal Properties of Some Short Chain Fatty Acids against Phytopathogenic Fungi

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植物病原菌에 대한 몇가지 低級脂肪酸의 抗菌特性

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ABSTRACT

The five short-chain fatty acids such as isobutyric(C-4), butyric(C-4), isovaleric(C-5), valeric(C-5) and caproic (C-6) acids obtained from the extract of common purslane showed wide antifungal spectra against spore germination and mycelial growth of the twenty five phytopathogenic fungi tested in vitro, although there were differences in antifungal potency among them. The antifungal potency of each fatty acid varied significantly against different fungi in spore germination and mycelial growth. The seventeen fungi used for spore germination test and the sixteen fungi used for mycelial growth test can be divided into three groups depending upon differences in minimal inhibitory concentration of each fatty acid for them, respectively. Caproic acid was significantly more toxic to germination than to mycelial growth of the test fungi, while the other four fatty acids did not show such a significant differences in toxicity with a few of exceptions as shown in valeric acid. The longer the chain-length of fatty acid was, the higher the antifungal potency was shown. The normal fatty acids such as butyric and valeric acid were more toxic than their isomers to spore germination and mycelial growth of the test fungi. Each fatty acid was more toxic to spore germination of obligate parasites and some of facultative parasites, and mycelial growth of facultative parasites than to spore germination and mycelial growth of saprophytes, respectively.

Key words: fatty acids, antifungal effect.

要 約

식미물 가식에서 얻은 5種의 低級脂肪酸인 isobutyric(C-4), butyric(C-4), isovaleric(C-5), valeric

(C-5), caproic (C-6) acids 등이 그들 사이에 抗菌力의 差異는 있었지만 供試한 25種의 植物病原菌의 胞子發芽나 菌糸生長에 대하여 幅넓은 抗菌스펙트럼을 보여 주었다. 各各의 脂肪酸의 抗菌力은 胞子發芽檢定이나 菌糸伸長檢定에 있어서 供試菌株에 따라 큰 差異를 보여 주었다. 胞子發芽檢定에 供試한 17種의 病原菌과 菌糸伸長檢定에 供試한 16種의 病原菌은 그들의 胞子發芽나 菌糸伸長을 제각기 完全히 阻害하는 各各의 脂肪酸의 最低阻害濃度(MIC)에 따라 3群으로 나눌 수 있다. 이밖에도 이 硏究의 結果에서 다음과 같은 假說을 提示할 수 있었다. 1) Caproic acid는 供試菌의 菌糸伸長보다 胞子發芽에 대하여 더욱 強한 毒性을 보였으나 약간의 例外는 있었지만 그 밖의 脂肪酸은 이와같은 毒性을 보이주지 않았다. 2) 脂肪酸의 사슬(鎖)이 길수록 抗菌力도 強하다. 3) n-Butyric acid와 n-Valeric acid는 그들의 異性體보다 抗菌力이 強하다. 4) 各各의 脂肪酸은 腐生菌의 胞子發芽나 菌糸伸長에 대한 것보다 活物寄生菌이나 몇가지 條件的 寄生菌의 그것들에 대하여 더욱 強한 毒性을 보여 주었다.

INTRODUCTION

We previously reported five antifungal fatty acids such as isobutyric, butyric, isovaleric, valeric and caproic acids which were isolated from the long-term storaged extract of common purslane(7,9). In the results of bioassay using *Alternaria alternata* Japanese pear pathotype, those fatty acids showed differences of antifungal potency each other and each fatty acid, likewise, showed different antifungal potency against the spore germination and the mycelial growth. The results also suggested that chain-length of these fatty acids might be related to their antifungal potency. There are some reports on the antifungal activities of fatty acid and their derivative, eventhough they are not bioproducts (4,5,10,12,13). On the other hand, some of fatty acids are known as growth stimulants and carbon sources for some phytopathogenic fungi(1,3). It has been well known that some of apple growers have used the common purslane extract for control of apple canker caused by *Valsa ceratosperma* in Korea since 1973, and antifungal potency of the extract was proved by the succeeding field and in vitro experiments(6). Those of fatty acids with continuous works on the antifungal potency and spectrum against various phytopathogenic fungi might find suitable application in plant disease control if they do not bring about chemical injury. The antifungal potency of these fatty acids merits attention with increased interest in fungicide system providing reduced environmental hazards.

Antifungal spectra of five short-chain fatty acids (C4-C6) against various phytopathogenic fungi, differencial antifungal potency of each fatty acid against mycelial growth and spore germination, and differences of antifungal potency related to chain-length of each fatty acid and parasitism of each fungus were presented in this paper.

MATERIALS AND METHODS

Fatty acids used. The following five short-chain fatty acids obtained through commercial source were used for *in vitro* tests: isobutyric acid ($C_4H_8O_2$), butyric acid ($C_4H_8O_2$), isovaleric acid ($C_5H_{10}O_2$), valeric ($C_5H_{10}O_2$) and caproic acid ($C_6H_{12}O_2$). Each fatty acid under test with a given concentration was incorporated in acetone and added to the media at 50°C. The control in these experiments was prepared similarly, but without the test fatty acid.

Phytopathogenic fungi used. The following phytopathogenic fungi collected from stock cultures of research organizations and infected plants in fields, were used to detect the minimum inhibitory concentration (MIC) of each fatty acids: *Gymnosporangium haraeanum, Puccinia kusanoi, Uromyces alopecuri, Melampsora coleosporioides, Ustilago nuda, Ustilago cynodontis, Venturia nashicola and Rhynchosporium secalis (Fields), Pellicularia sasaki, Rhizoctonia solani, Colletotrichum nigrum, Botrytis cinerea, Aspergillus niger, Penicillium digitatum and*

Rhizopus stolonifer (stock cultures of Tottori University), *Phytophthora capsici*, *Alternaria alternata* Japanese pear pathotype, *Fusarium roseum* f.sp. *cerealis* and *Fusarium oxysporum* f. sp. *cepae* stock cultures of Nagoya University), *Glomerella cingulata*, *Cochliobolus miyabeanus* and *Mycosphaerella pinodes* (stock culture of Okayama University), *Valsa ceratosperma* (stock culture of Chungnam University), *Pyricularia oryzae* (stock culture of Kyushu University) and *Phomopsis fukushi* (stock culture of Tottori Fruit Expt. Sta.). These test fungi include obligate parasites, facultative parasites and saprophytes. All of the obligate parasites and some of selected facultative and saprophytic parasites forming abundant spores on potato sucrose agar medium (PSA) were used for MIC tests against their spore germination, while all of facultative and saprophytic parasites were used for MIC tests against their mycelial growth.

Culture media. Water agar (2%) for MIC tests against conidia germination and potato sucrose agar against mycelial growth of each test fungus were prepared, respectively. Each medium was autoclaved and cooled to approximately 50°C in a water bath. Each medium was finally prepared to contain a given concentration of each fatty acid with 5% of acetone as a dispersant previously reported(8). No attempt was made to control pH of each medium.

Preparation of inocula. Spores of each test fungus was prepared as follows: spore mass on the infected plants and spore-forming culture on PSA in petri dishes were harvested by blushing and suspending in distilled water, then sieved through a few of layers of cheese-cloth and adjusted to give the final concentration which was determined microscopically to observe 20-30 spores/field at 100 x. On the other hand, mycelial mat of each test fungus was prepared from 5-7 day-old cultures on PSA at 26°C.

Determination of MIC. Each MIC of the test fatty acid against spore germination and mycelial growth of each test fungus was determined by the usual dilution plate method. Each spore suspension prepared was seeded on water agar containing a given concentration of each fatty acid by a glass atomizer, incubated for 24 hr at 26°C. MIC of each fatty acid was then determined microscopically by observation of the spore germination which was completely inhibited at the lowest concentration in the range of 10-300 ppm. On the other hand, 4 mm disc prepared from the mycelial mats of each test fungus was seeded on PSA containing a given concentration of each fatty acid, incubated for 5 days at 26°C and then MIC of each fatty acid was determined by observation of the mycelial growth which was completely inhibited at the lowest concentration in the range of 10-500 ppm. No one of MIC tests of each fatty acid at higher concentration than 500 ppm was considered.

RESULTS AND DISCUSSIONS

Antifungal spectra of fatty acids against spore germination. Each of fatty acids tested showed a wide antifungal spectrum against the seventeen test fungi, though there were differences of antifungal potency among the five fatty acids as shown in Table 1. The results also showed that antifungal potency of each fatty acid varied significantly according to the different fungi. The seventeen fungi tested can be divided into three groups depending upon MIC of each fatty acid for their spore germination. Spore germination of the fungi included in the first group, the second group and the third group were completely inhibited at concentrations lower than 100 ppm, between 100 and 300 ppm, and higer than 300 ppm of each fatty acids, respectively. Of the seventeen fungi tested, *Gymnosporangium haraeanum*, *Puccinia kusanoi*, *Uromyces alopecuri*, *Melampsora coleosporioides*, *Ustilago cynodontis*, *Ustilago nuda* and *Rhynchosporium secalis* in the antifungal spectra of all the five fatty acids, *Venturia nashicola* and *Pyricularia oryzae* in the antifungal spectra of valeric and caproic acids, and *Phytophthora capsici*, *Glomerella*

Table 1. Minimal inhibitory concentration (MIC) of fatty acids against conidia germination of selected phytopathogenic fungi on water agar.

Fungus	MIC (ppm) of fatty acids[a]				
	IBA	NBA	IVA	NVA	NCA
Gymnosporangium haraeanum (aeciospore)	50ppm	70	80	50	30
Puccinia kusanoi (uredospore)	80	70	80	50	70
Uromyces alopecuri (uredospore)	80	70	70	50	40
Melampsora coleosporioides (uredospore)	50	70	50	40	30
Ustilago cynodontis	50	40	50	40	40
Ustilago nuda	80	50	80	50	60
Phytophthora capsici (zoospore)	280	150	150	150	90
Rhizopus stoloniter	> 300	> 300	> 300	> 300	250
Glomeralla cingulata (conidiospore)	300	170	200	180	80
Cochliobolus miyabeanus (conidiospore)	300	180	200	170	80
Venturia nashicola (conidiospore)	270	120	120	70	40
Pyricularia oryzae	280	120	100	50	30
Rhynchosporium secalis	80	60	60	50	40
Alternaria alternata Japanese pear pathotype	300	230	230	170	50
Aspergillus niger	> 300	> 300	> 300	> 300	> 300
Penicillium digitatum	> 300	> 300	> 300	200	150
Fusarium oxysporum f. sp. *cepae* (microconidium)	> 300	270	> 300	250	230

[a] IBA = isobutyric acid, NBA = butyric acid, IVA = isovaleric acid, NVA = valeric acid NCA = caproic acid

cingulata, *Cochliobolus miyabeanus* and *Alternaria alternata* Japanese pear pathotype in the antifungal spectrum of caproic acid were included in the first group and their spore germination were completely inhibited at concentrations between 30 and 80 ppm of the corresponding fatty acid. *Phytophthora capsici*, *Glomerella cingulata*, *Cochliobolus miyabeanus* and *Alternaria alternata* Japanese pear pathotype in the antifungal spectra of the other four fatty acids except caproic acid, *Venturia nashicola* and *Pyricularia oryzae* in the antifungal spectra of isobutyric, butyric and isovaleric acids, *Fusarium oxysporum* f. sp. *cepae* in the antifungal spectra of butyric, valeric and caproic acids, *Penicillium digitatum* in the antifungal spectra of valeric and caproic acids, and *Rhizopus stolonifer* in the antifungal spectrum of caproic acid were included in the second group and their spore germination were completely inhibited at concentrations between 100 and 300 ppm of the corresponding fatty acid. *Aspergillus niger* in the antifungal spectra of all the five fatty acids, *Rhizopus stolonifer* in the antifungal spectra of the other four fatty acids except caproic acid, *Penicillium digitatum* in the antifungal spectra of isobutyric, butyric and isovaleric acids, and *Fusarium oxysporum* f. sp. *cepae* in the antifungal spectra of isobutyric and isovaleric acids were included in the third group and their spore germination were not completely inhibited at concentrations lower than 300 ppm of the corresponding fatty acid.

Antifungal spectra of fatty acids against mycelial growth. Each of fatty acid separately tested also showed a wide antifungal spectrum against mycelial growth of the sixteen test fungi, though there were differences of antifungal potency among the five

Table 2. Minimal inhibitory concentrations (MIC) of fatty acids against mycelial growth of selected phytopathogenic fungi on potato sucrose agar.

Fungus	MIC (ppm) of fatty acids[a]				
	IBA	NBA	IVA	NVA	NCA
Phytophthora capsici	450	230	180	200	130
Rhizopus stolonifer	> 500	> 500	> 500	> 500	> 500
Glomerella cingulata	> 500	220	270	190	180
Cochliobolus miyabeanus	> 500	250	320	200	180
Mycosphaerella pinodes	> 500	450	400	280	230
Valsa ceratosperma	> 500	300	280	200	200
Phomopsis fukushi	370	450	350	250	430
Colletotrichum nigrum	300	180	250	180	180
Botrytis cinerea	> 500	400	350	350	200
Pyricularia oryzae	350	180	180	180	130
Alternaria alternata Japanese pear pathotype	450	320	280	250	230
Aspergillus niger	> 500	> 500	> 500	> 500	> 500
Penicillum digitatum	> 500	> 500	> 500	> 500	> 500
Fusarium roseum f. sp. *cerealis*	> 500	300	250	250	170
Pellicularia sasaki	450	170	450	200	190
Rhizoctonia solani	300	150	230	180	130

[a] IBA = isobutyric acid, NBA = butyric acid, IVA = isovaleric acid, NVA = valeric acid, NCA = caproic acid.

fatty acids as shown in Table 2. The results also showed that antifungal potency of each fatty acid varied significantly against different fungi. No one of the five fatty acids completely inhibited mycelial growth of the test fungi at concentrations lower than 100 ppm. Thus, it was presumed that those of antifungal fatty acids were more active against spore germination than mycelial growth of the test fungi. The sixteen fungi tested can be divided into mycelial growth of the test fungi. The sixteen fungi tested can be divided into three groups depending upon MIC of each fatty acid for their mycelial growth. Mycelial growth of the fungi included in the first group, the second group and the third group were completely inhibited at concentrations between 100 and 300 ppm, between 300 and 500 ppm and higer than 500 ppm of each fatty acid, respectively. Of the sixteen fungi tested, *Colletotrichum nigrum* and *Rhizoctonia solani* in the antifungal spectra of all the five fatty acids, *Phytophthora capsici, Glomerella cingulata, Cochliobolus miyabeanus, Valsa ceratosperma, Pyricularia oryzae* and *Fusarium roseum* f. sp. *cerealis* in the antifungal spectra of the other four fatty acids except isobutyric acid, *Pellicularia sasaki* in the antifungal spectra of butyric, valeric and caproic acids, *Alternaria alternata* Japanese pear pathotype in the antifungal spectra of isovaleric, valeric and caproic acids, *Mycosphaerella pinodes* in the antifungal spectra of valeric and caproic acids, *Phomopsis fukushi* in the antifungal spectrum of valeric acid, and *Botrytis cinerea* in the antifungal spectrum of caproic acid were included in the first group and their mycelial growth completely inhibited at concentrations between 100 and 300 ppm of the corresponding fatty acid. *Phytophthora capsici* in the antifungal spectrum of isobutyric acid, *Mycosphaerella pinodes* in the antifungal spectra of butyric and isovaleric acids, *Phomopsis fukushi* in the antifungal spectra of the other four fatty acids except valeric acid, *Botrytis cinerea* in the antifungal spectra of butyric, isovaleric and valeric acids, *Pyricularia oryzae* in the antifungal spectrum of isobutyric acid, *Alternaria alternata* Japanese pear pathotype in the antifungal spectra of isobutyric and butyric acids, and *Pellicularia sasaki* in the

antifungal spectra of isobutyric and isovaleric acids were included in the second group and their mycelial growth were completely inhibited at concentrations between 300 and 500 ppm of the corresponding fatty acid. *Rhizopus stolonifer*, *Aspergillus niger* and *Penicillium digitatum* in the antifungal spectra of all the five fatty acids and *Glomerella cingulata*, *Cochliobolus miyabeanus*, *Mycosphaerella pinodes*, *Valsa ceratosperma*, *Botrytis cinerea* and *Fusarium roseum* f. sp. *cerealis* in the antifungal spectrum of isobutyric acid were included in the third group and their mycelial growth were not completely inhibited at concentrations lower than 500 ppm of the corresponding fatty acid.

Differences of MIC of each fatty acid for spore germination and mycelial growth. From the data in Table 1 and 2, differences of MIC of each fatty acid for spore germination and mycelial growth of the selected eight fungi were found. Without exceptions, all the five antifungal fatty acids were more active against spore germination than against mycelial growth of the selected eight fungi such as *Phytophthora capsici*, *Rhizopus stolonifer*, *Glomerella cingulata*, *Cochliobolus miyabeanus*, *Pyricularia oryzae*, *Alternaria alternata* Japanese pear pathotype, *Aspergillus niger* and *Penicillium digitatum*. Caproic acid was approximately two to four times more toxic to spore germination than to mycelial growth of all the eight test fungi and the other four fatty acid were slightly more toxic to spore germination than to mycelial growth of all the eight test fungi except *Pyricularia oryzae* and *Penicillium digitatum* to valeric acid. As described later, antifungal potency of caproic acid against both of spore germination and mycelial growth of the test fungi generally was the most great. It is not certain whether such difference is due to chemical character of the test fatty acids or due to the media used. It might be originated from sugar in PSA for the mycelial culture (2).

Relationship between antifungal potency and chain-length of fatty acids. With a few of exceptions, the data in Table 1 and 2 suggested that the longer the chain-length of fatty acid was, the higher the antifungal potency was shown. The five fatty acids tested showed higher antifungal potency against spore germination and mycelial growth of most the test fungi in order of isobutyric (C-4) butyric (C-4) and isovaleric (C-5), valeric (C-5) and caproic (C-6) acids with increase of chain-length. In a paper recently presented, it was reported that fatty acids of chain-length C8-C10 were more active than those of chain-length C5-C7, against spore germination of some wood-decay fungi(12). In the present study, differences of antifungal potency between normal fatty acids and their isomers with the same chain-length were also recognized. With a few of exceptions, higher concentrations of isobutyric and isovaleric acids than their normal acids were required to inhibit completely both of spore germination and mycelial growth of the test fungi.

Relationship between antifungal potency of fatty acids and parasitism of fungi. The data in Table 1 and 2 also suggested that antifungal potency of each fatty acid tested was related to parasitism of the test fungi. In general, all the fatty acids tested were more toxic to spore germination of all the four obligate parasites and the three facultative parasites than to that of all the three saprophytes and the seven facultative parasites. Such a relation was also recognized in toxicity of each fatty acid against mycelial growth of the test fungi. All the fatty acids tested were more toxic to mycelial growth of facultative parasites than that of saprophytes tested. Of the five fatty acids tested, caproic acid could not completely inhibit spore germination of *Aspergillus niger* at concentrations lower than 300 ppm and mycelial growth of *Rhizopus stolonifer*, *Aspergillus niger* and *Penicillium digitatum* at concentrations lower than 500 ppm in spite of its greatest antifungal potency.

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