Investigation of Antifungal Activity for Plant Disease Control by Compost Teas Fermented under Different Temperatures

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식물병 관리를 위해 다양한 온도조건에서 발효한 퇴비차의 항진균 활동에 관한 연구

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Abstract

Efficacy of antifungal activity on plant pathogens by compost teas fermented under different temperatures was studied. Compost teas are recently chosen by agricultural producers for the better method of controlling plant diseases under increase of public consciousness against use of chemicals for controlling the diseases. Compost tea has been intensively studied; however, understanding of compost tea is still not well developed, and temperature influence during fermentation of compost tea on its antifungal activity has not been investigated. In this study, antifungal activities of compost teas fermented at 10, 20, 30, and 40°C against selected 10 pathogens were observed. From the results, antifungal activities of compost teas at 20 and 30°C of fermentation-temperatures showed the strongest while the weakest activity was observed with the compost tea at 10°C. Change of the activity by the fermentation-temperature apparently implied that microbes in the compost tea were strongly involved in its antifungal activity.

keywords : Aerated compost tea, Antifungal activity, Fermentation-temperature, Plant disease

1. Introduction

Because of surplus production and limited use of compost under the current social situation in Japan, compost needs to have other applications rather than use only as fertilizer (Tateda et al., 2007). Use of compost tea has been reported as a promised practice for suppressing some plant diseases. Since public concern over chemical control of plant disease has increased (McQuilken et al., 1994), agricultural producers are interested in non-chemical control of plant disease and choose compost tea instead of using chemicals such as fungicide (Ingram and Millner, 2007).

Mechanisms of the suppression against plant pathogens by compost tea are described the following four patterns; nutrient and space competition between plant pathogens and microbes in compost tea, production of antibiotics from microbes in compost tea, direct parasitism on pathogens by microbes in compost tea, and stimulation of defense systems naturally possessed by plants (Scheuerell, 2003). Compost tea has been intensively studied by many researchers (Scheuerell and Walter, 2002), however, despite of the intensive studies on compost tea, understanding and effective application of compost tea is still under development.

Temperature is one of the most influencing factors to microbes' activity for making compost. Tateda et al. (2002) reported an important role of temperature in compost production. Temperature of 20°C was critical for start-up of composting operation and compost start-up was very fast when ambient temperature was above 20°C and the start-up became very sluggish at the ambient temperature below 20°C. Factor of temperature is extremely important for microbes' activity; however, influence of antifungal activity of compost tea fermented under different temperatures has not been studied yet. Scheuerell and Walter (2002) also pointed out no reports, so far, describing the effects of fermentation temperature on antifungal activity of compost tea.

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The purpose of this study was investigation of antifungal activity of compost teas fermented under different temperatures. From the result of the previous study (Tateda et al., 2007), it was found that the compost tea produced under one-day aeration showed relatively better efficacy against selected plant pathogens among other options, therefore, compost tea under one-day aeration was used in this study.

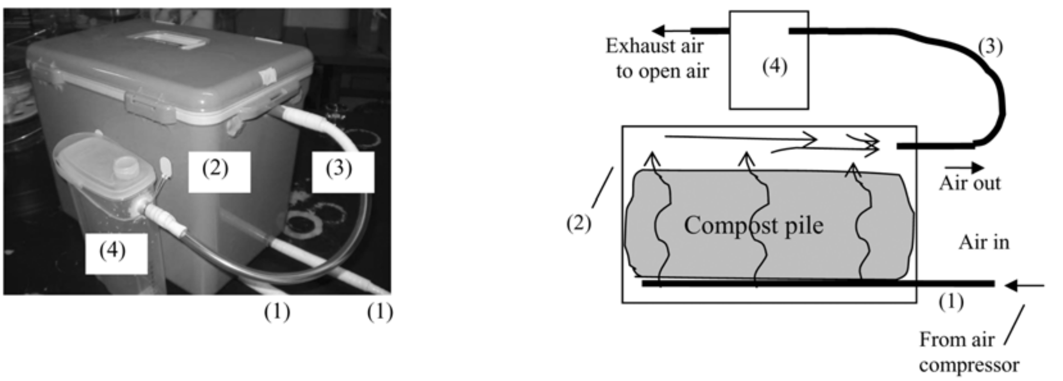
2. Materials and Methods

2.1. Composts

Dog food (Aijo-Monogatari Beef taste, Yeaster, Japan) was used as organic substance for composting. The calorie of the dog food was more than 290 kcal/100 g and ingredients were as follows; protein (>20.0%), fat (>5.0%), fibers (<6.0%), ash (<10.0%), calcium (>1.0%), phosphorus (>0.8%), salt (>0.5%), linoleic acid (>1.0%), vitamin A (>5,000 IU/kg), vitamin B_1 (>1.0 mg/kg), vitamin B_2 (>2.2 mg/kg), biotin (>0.1 mg/kg), and others such as potassium, magnesium, iron, copper, and so on. Compost was produced in the composting vessel with forced air supply system shown in Fig. 1 (Ejiri and Igarashi, 2003). The dog food was grinded and powdered by a home juicer mixer. The powdered dog food and seed compost were mixed with the ratio of 1:1 in weight. The water content of the mixed material was adjusted to 50~55% with tap water. Composting was conducted with 9 L/min air supply. Temperature at the mid part of the compost pile was measured during composting for ensuring composting performance. Compost after 10 day-operation was used for making compost tea.

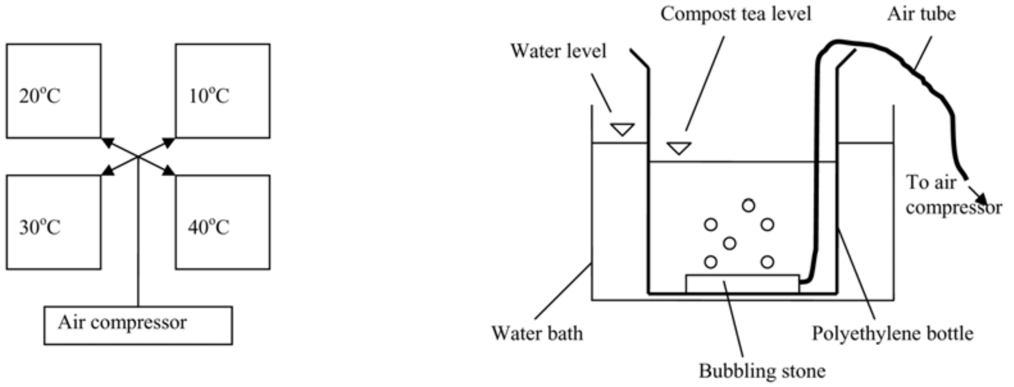
2.2. Compost tea

Tap water was used for the solution for making compost tea. The compost prepared by the process described in the previous section was mixed with tap water at the ratio of 1:10 in volume in polyethylene bottles. The four bottles were then placed into water baths in which water temperature was adjusted to 10, 20, 30, and 40°C and each bottle was aerated for one day (Fig. 2). After one day, aeration was stopped and let compost settled down to the bottom of the bottles. Then, compost teas (supernatant) were sampled for experiments.



(a) Reactor appearance (b) Description of reactor

Fig. 1. The compost reactor.
(1) Pipes for supplying air into the reactor, (2) Body of reactor, (3) Tube for collecting exhaust air from reactor, and (4) Collection tank for evaporated compost water. Arrows show air flow direction.



(a) Configuration of water bathes (b) Experimental system for fermentation of compost tea

Fig. 2. Experimental sketches for fermentation of compost tea.

Table 1. Pathogens used in this study

Pathogens	Plant disease caused by the pathogen
*Fusarium proliferatum**	Fusarium wilt
Botrytis tulipae MAFF237888	Botrytis blight
Fusarium oxysporum f. sp. *tulipae* MAFF305609	Tulip bulb rot
*Pyricularia oryzae**	Rice blast
Cercospora kikuchii MAFF305041	Purple stain
*Colletotrichum dematium**	Anthracnose
Phytophthora cactorum MAFF235096*	Tulip blossom blight
*Alternaria brassicicola**	Black spot
*Phytophthora capsici**	Late blight
Sclerotinia sp.*	Cottony rot, white mold

MAFF: Ministry of Agriculture, Forestry and Fisheries, *: The sources were stated in Acknowledgement.

2.3. Plant pathogens

The plant pathogenic fungi of plant disease shown in Table 1 were selected in order to verify antifungal activity of compost teas. Potato dextrose agar (Kanto Kagaku, Japan) was used as a growth medium for the fungi.

2.4. Experiment for observing antifungal activity of compost teas

Agar Dilution Method was employed for evaluating antifungal activity of the compost teas fermented under different temperatures. Potato dextrose agar (19.5 g) was dissolved into 500 mL of pure water, and then the mixed solution was provided to prepare 45, 49.5, 49.95, and 50 mL in erlenmeyer flask. Each erlenmeyer flask was closed with aluminium foil and autoclaved (120ºC, 20 minutes). After autoclaved, each erlenmeyer flask was cooled down to about 40ºC and then 0, 0.05, 0.5, and 5 mL of compost tea were mixed into 50, 49.95,49.5, and 45 mL of agar in the erlenmeyer flask, respectively, under sterilized condition in order to prepare control (no compost tea), 10, 100, and 1000 times diluted compost tea agar media. A 10 mL of each medium was pored into sterilized petri dishes and the process was done in triplicates. After agar media in the petri dishes were solidified, the colony disk of the pathogenic fungi incubated for one-week were transferred into the petri dishes under the sterilized condition. Evaluation of antifungal activity by compost teas was done by visual observation based on hyphal growth degree on each agar.

3. Results and Discussion

Table 2. Representative data for antifungal activity of compost teas fermented in different temperatures

Fer. Temp	Dilu-. tion	Pathogens and Observation days (1st, 3rd, and 5th days)																													
		Fusarium proliferatum			*Botrytis tulipae*			*Fusarium oxysporum* f. sp. *tulipae*			*Pyricularia oryzae*			*Cercospora kikuchii*			*Colletotrichum dematium*			*Phytophthora cactorum*			*Alternaria brassicicola*			*Phytophthora capsici*			*Sclerotinia* sp.		
		1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5	1	3	5
10 °C	C	I	III	III	I	III	IV	I	III	III	-	I	I	-	II	II	-	II	II	I	II	III	I	III	III	-	I	I	II	V	V
	10^{-1}	-	I	I	I	III	III	I	II	II	-	-	-	-	I	I	-	I	I	I	II	II	-	I	I	-	I	I	II	II	II
	10^{-2}	-	II	II	I	III	III	I	II	II	-	I	I	-	I	I	-	I	I	-	II	II	-	I	II	-	I	-	II	V	I
	10^{-3}	I	III	III	I	III	IV	I	III	III	-	I	I	-	I	I	-	I	II	I	II	II	-	I	II	-	I	I	II	V	V
20 °C	C	I	III	IV	I	IV	IV	I	III	III	-	II	I	-	I	I	-	II	II	I	II	II	I	III	III	-	I	I	II	V	V
	10^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-2}	-	I	I	I	I	I	-	I	I	-	-	-	-	-	-	-	-	I	-	I	I	-	-	I	-	I	I	II	I	I
	10^{-3}	-	I	I	I	I	I	I	I	I	-	-	-	-	-	-	-	I	II	-	I	I	-	I	I	-	I	I	II	I	I
30 °C	C	I	III	III	I	III	IV	I	III	III	-	I	I	-	I	I	-	II	II	-	II	II	I	III	III	-	I	I	II	V	V
	10^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10^{-2}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	-	-	-	-	-	I	-	-	-	-	-	-
	10^{-3}	-	II	III	I	II	III	I	III	III	-	I	I	-	I	-	-	II	II	-	II	II	-	I	II	-	I	I	II	IV	V
40 °C	C	I	III	III	I	IV	IV	I	III	III	-	I	I	-	I	I	-	I	I	-	II	I	I	III	III	-	I	I	II	V	V
	10^{-1}	-	-	I	-	-	-	-	I	I	-	-	-	-	-	-	-	-	-	-	I	I	-	-	-	-	-	-	-	-	I
	10^{-2}	I	III	III	I	III	IV	I	III	III	-	I	I	-	I	I	-	I	I	-	II	I	-	III	III	-	I	I	II	V	V
	10^{-3}	I	III	III	I	IV	IV	I	III	III	-	I	I	-	I	I	-	I	I	-	II	I	-	III	III	-	I	I	II	V	V

Note: Roman numerals I to V show the growth levels of fungi on the medium. III means standard growth of fungi on the medium. IV and V mean more growth than standard and I and II mean less growth than III. The less the numeral is, the stronger the antifungal activity is. -: no growth of the fungus on the medium. C: control.

Results of antifungal activity of compost teas fermented under different temperatures on the selected pathogens were shown in Table 2. According to the table, the control samples of most of pathogens showed the level of standard growth, so it could be said that plant pathogens selected here were active. At 10°C of fermentation-temperature, very week but some antifungal activities on the specific pathogens such as *Fusarium proliferatum*, *Alternaria brassicicola*, and *Sclerotinia* sp. were shown when 1/10 dilution compost tea was used; however, the compost tea did not always show any antifungal activity against the rest of the pathogens. At 20°C, the 1/10 compost tea showed very strong antifungal activities on all the pathogens. Antifungal activity became slightly weaker on all pathogens when the 1/100 compost tea was used and the same level of the activity was kept when 1/1000 compost tea was applied. At 30°C, the both 1/10 and 1/100 compost teas showed strong antifungal activities on all the pathogens. However, the antifungal activity disappeared when 1/1000 compost tea was applied and the growth of the pathogens became the same as the one of the control. At 40°C, strong antifungal activities on all the pathogens were shown with the 1/10 compost tea, whereas, the antifungal activities on all the pathogens disappeared with the 1/100 and 1/1000 compost teas.

Except for the case of 10°C of fermentaion-temperature, the antifungal activity of compost teas showed the same efficacy against all the pathogens under the respective fermentation-temperatures. This result verified that the antifungal activity of the compost tea was not selective to the specific pathogens on plant foliage (*Botrytis tulipae*, *Pyricularia oryzae*, *Cercospora kikuchii*, *Colletotrichum dematium*, *Alternaria brassicicola*, and *Sclerotinia* sp.) and in soil (*Fusarium proliferatum*, *Fusarium oxysporum* f. sp. *tulipae*, *Phytophthora cactorum*, and *Phytophthora capsici*) when compost tea was produced above 20°C of fermentation-temperature. The fact of different antifungal activity trends shown below and above 20°C may be related to the critical temperature for microbes' activities (Tateda et al., 2002). The highest antifungal activity was shown with the compost tea at 30°C of fermentation-temperature, and the secondly highest antifungal activity was seen with the compost tea at 20°C. The antifungal activity of the compost tea at 40°C came next after that of 20°C, and the lowest activity was observed with the compost tea at 10°C of fermentation-temperature. Comparing antifungal activity of between compost teas at 20°C and 30 and 40°C of fermentation-temperatures, different trends of the activity were found. Virtually no growth of pathogens were found on the 1/10 and 1/100 at 30°C and 1/10 at 40°C compost tea media, however, antifungal activities were not observed with the 1/1000 and the 1/100 and 1/1000 compost teas, respectively, at all. At 20°C, the antifungal activity became weak when the 1/100 compost tea was used comparing to the activity when the 1/10 compost tea was used, and remarkably the level of activity was maintained with the 1/1000 compost tea. Sudden disappearance of antifungal activity was not observed at this temperature as shown the tests at 30 and 40°C of fermentation-temperature. The reason of the sudden disappearance of antifungal activity along with dilution of compost teas could not be known in this study and it was found that antifungal activity of 20°C of fermentation-temperature lasted longer along dilution of compost teas. With use of 1/10 dilution compost tea, high antifungal activity can be expected at 20, 30, and 40°C of fermentation-temperatures against all the pathogens selected in this study. Change of antifungal activity on the pathogens based on fermentation-temperatures implied that antifungal activity of compost tea mainly attributed to biological aspect and microbes in the compost tea played an essential role for suppressing plant disease. Since the results presented here were in vitro base, in vivo tests for confirming the antifungal activity by compost tea should be needed but it can be said that one of basic knowledge for producing compost tea was obtained by this study.

4. Conclusions

Following conclusions are drawn from this study.

1) The high antifungal activities were shown with the compost teas at 20 and 30°C of fermentation-temperatures. The weakest activity was shown with the compost tea at 10°C of fermentation-temperature.
2) Except for 10°C of fermentation-temperature, the 1/10 dilution compost tea, the lowest dilution, at 20, 30, and 40°C of fermentation-temperatures showed the highest antifungal activities on all the selected pathogens in this study.
3) The antifungal activity of 20°C of fermentation-temperature would last in further dilution, whereas, the activity of 30 and 40°C of fermentation-temperature would disappear in further dilution.
4) Microbes in compost tea may strongly play an essential role against plant pathogens.

요 약

본 논문에서는 다양한 온도조건에서 발효한 퇴비차의 항진균 효능에 관한 연구내용을 제시하였다. 최근에 많은 농가에서 일반 대중의 친환경 농산물에 대한 선호에 부응하

고 식물병 예방을 위하여 퇴비차를 선택하고 있다. 퇴비차에 대한 많은 연구가 진행되었으나 아직 퇴비차에 대한 이해가 부족하고 퇴비차가 발효되는 동안 온도가 항진균 효과에 미치는 영향에 대한 연구는 없었다. 본 연구에서는 10, 20, 30, 40°C에서 발효한 퇴비차의 항진균 활동이 본 연구에서 선정한 10종의 병원성 미생물에 미치는 영향을 관찰하였다. 결과에 따르면 20에서 30°C에서 발효한 퇴비차의 항진균 활동이 가장 강한 것으로 나타났으며 10°C에서 발효한 퇴비차가 가장 약한 것으로 밝혀졌다. 발효온도가 항진균 활동에 미치는 변화는 퇴비차의 미생물이 항진균 활동에 강하게 개입하고 있음을 시사해주고 있다.

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