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SHORT COMMUNICATION

Tomoo Hosoe · Koohei Nozawa · Ken-ichi Kawai
Yuichi Yamaoka · Keizo Katsuya

Teliospore-inducing activity for wheat leaf rust in the extracts of various host plant leaves infected with telia of rust fungi

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Abstract MeOH and water extracts were obtained from 16 species of infected leaves with rust fungi belonging to 18 species in 6 families: Pucciniaceae, Melampsoraceae, Coleosporiaceae, Pileolariaceae, Phragmidiaceae, and Phakopsoraceae. All the extracts of rust-infected plants with telia showed the teliospore-inducing activity for wheat leaf rust (*Puccinia recondita* f. sp. *tritici*).

Key words Rust fungus · Teliospore-inducing substances · Teliospore production · Wheat leaf rust

Rust fungi (Uredinales) produce up to five kinds of spore stages in their life cycle. They repeatedly produce urediniospores on their host plants under favorable conditions, but generally produce teliospores only once when the plants approach maturity (Takahashi et al. 1965; Agrios 1997). Teliospores, also known as commonly "resting" or "winter" spores, are capable of surviving unfavorable conditions for long periods of time (Cummins and Hiratsuka 2003). We previously reported that substances inducing teliospore production of *Puccinia recondita* Roberge ex Desm. f. sp. *tritici* Eriksson are found in rust-infected wheat leaves with numerous telia collected at harvest time. Active substances were purified and showed activity at 2 ng/ml. We estimated that these acidic substances are smaller than 1000 MW in size, but the chemical structures remain to be determined (Hosoe et al. 2001).

T. Hosoe (✉) · K. Kawai
Faculty of Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan
Tel. +81-3-5498-5789; Fax +81-3-5498-5789
e-mail: hosoe@hoshi.ac.jp

K. Nozawa
School of Pharmaceutical Sciences, Ohu University, Fukushima, Japan

Y. Yamaoka · K. Katsuya
Graduate School of Life & Environmental Sciences, University of Tsukuba, Ibaraki, Japan

The role of host plants infected with rust fungi teliospores of 1 species in the induction of teliospore formation of other species has yet to be elucidated. Therefore, in this study we sought to determine the presence of substances influencing teliospore production of *P. recondita* f. sp. *tritici* in 17 species of host plant infected with 18 species of rust fungi.

Nineteen samples of rust-infected host plant leaves with teliospores, such as wheat infected with *Puccinia recondita* f. sp *tritici*, were collected in 1999 and 2000 (Table 1). These plants, belonging to 15 genera and 9 families, were infected by 19 species of rust fungi belonging to 7 genera and 6 families. All the samples except no. 11 were collected in fields, air-dried, and stored in a refrigerator until use. Potted plants of *Chrysanthemum morifolium* Ramat. infected with *Puccinia horiana* Henn. were obtained from a flower shop. No. 11 plants were repeatedly inoculated with basidiospores of the rust fungus to acquire adequate sample volume. Samples leaves, except for nos. 1, 6, 10, and 17, possessed abundant teliospores. Because of the length of time required for leaves of *Populus nigra* L. var. *italica* Du Rai (no. 3) and *Salix sachalinensis* Fr. Schm. (no. 7) to fall, these samples were dark brown in color when collected. In contrast, leaves of *Aster glehni* Fr. Schm. var. *hondoensis* Kitam. (no. 1), *Petasites japonicus* Miq. (no. 2), *Populus nigra* L. var. *italica* (no. 4), *Salix chaenomeloides* Kimura (no. 6), and *Lysimachia clethroides* Duby (no. 17) were greenish colored upon collection.

Extraction and separation processes are summarized in Fig. 1. Leaves were air-dried, crushed into small pieces, and extracted with dichloromethane (CH_2Cl_2). Each defatted residue was extracted twice with methanol (MeOH) and then water for 24 h. After removal of leaves by filtration, each extract was evaporated at reduced pressure. The MeOH and water extracts from each sample were diluted with 10 ml water, then acidified to pH 2 with 4 M HCl and extracted with ethyl acetate (AcOEt) (30 ml each, three times). The AcOEt layer was dried over sodium sulfate and evaporated at reduced pressure until dry. Each residue was suspended in 5 ml water, then centrifuged (f = 3850 *g*, 20 min). The supernatant was separated by chromatography

Table 1. List of rust-infected plant leaves with abundant telia

No.	Sample no.	Rust family	Rust species	Host species (family)	Date collected	Location[a]	Dry weight (g)
15	1	Colesporiaceae	*Coleosporium asterum* (Dietel) P. et H. Sydow	*Aster glehni* Fr. Schm. var. *hondoensis* Kitam. (Compositae)	29-Sept-00	A	24
13	2		*C. yamabense* (Saho) Hiratsuka, f.	*Petasites japonicus* Miq. (Compositae)	29-Sept-00	A	18
14	3	Melampsoraceae	*Melampsora abietis-populi* S. Imai	*Populus nigra* L. var. *italica* Du Rai (Salicaceae)	6-Nov-00	B	70
16	4		*M. larici-populina* Klebahn	*Populus nigra* L. var. *italica* Du Rai (Salicaceae)	15-Oct-99	C	56
17	5		*M. populnea* (Persoon) Karsten	*Populus sieboldi* Miq. (Salicaceae)	14-Oct-00	D	64
12	6		*M. chelidonii-pierotii* Matsumoto	*Salix chaenomeloides* Kimura (Salicaceae)	28-Oct-00	E	48
11	7		*M. epiphylla* Dietel	*S. sachalinensis* Fr. Schm. (Salicaceae)	1-Nov-00	F	94
18	8	Phakosporaceae	*Phakopsora pachyrhizi* H. et P. Sydow	*Pueraria lobata* (Wild.) Ohwi (Leguminosae)	2-Dec-00	G	38
19	9	Phragmidiacae	*Phragmidium pauciloculare* (Dietel) H. et P. Sydow	*Rubus parvifolius* L. var. *triphyllus* Nakai (Rosaceae)	2-Dec-00	G	20
6	10	Pileolariaceae	*Pileolaria klugkistiana* (Dietel) Dietel	*Rhus javanica* L. (Anacardiac)	18-Nov-00	G	86
7	11	Pucciniaceae	*Puccinia horiana* Henn.	*Chrysanthemum morifolium* Ramat. (Compositae)	29-Oct-01	H	2.6
3	12		*P. tanaceti de Candolle* var. *tanaceti*	*Chrysanthemum morifolium* Ramat. (Compositae)	5-Dec-00	G	30
4	13		*P. miscanthi* Miura	*Miscanthus sinensis* Anderss. (Gramineae)	29-Sept-00	A	54
5	14		*P. magnusiana* Koernicke	*Phragmites longivalvis* Steud. (Gramineae)	28-Oct-00	E	72
2	15		*P. recondita* f. sp. *tritici*	*Triticum aestivum* L. (Gramineae)	6-Jun-00	I	20
1	16		*P. polygoni-amphibii* Persoon var. *tovariae* Arthur	*Polygonum cuspidatum* Sieb. et Zucc. (Polygonaceae)	29-Sept-00	A	34
10	17		*P. dieteliana* P. Sydow ex Dietel	*Lysimachia clethroides* Duby (Primulaceae)	28-Oct-00	J	20
9	18		*P. tokyensis* P. et H. Sydow	*Cryptotaenia japonica* Hassk. (Umbelliferae)	1-Nov-00	K	14
8	19		*Uromyces lespedezae-procumbentis* (Schweinitz) Curtis var. *lespedezae-procumbentis*	*Lespedeza bicolor* Turcz. (Leguminosae)	29-Sept-00	A	34

[a] Locations are as follows: A, Sugadaira Montane Research Center, University of Tsukuba in Nagano; B, Experimental Station of Tokyo University at Tanashi in Tokyo; C, The Tokyo University Forest at Furano in Hokkaido; D, Makibaen of Koiwai Farm at Shizukuishi in Iwate; E, Bank of Kokai River at Shimozuma in Ibaraki; F, Yumihari Pass at Nikko in Tochigi; G, Campus of The University of Tsukuba in Ibaraki; H, flower shop at Tsukuba in Ibaraki; I, National Agricultural Research Center at Tsukuba in Ibaraki; J, Mt. Tsukuba in Ibaraki; K, Mt. Ogura at Nikko in Tochigi

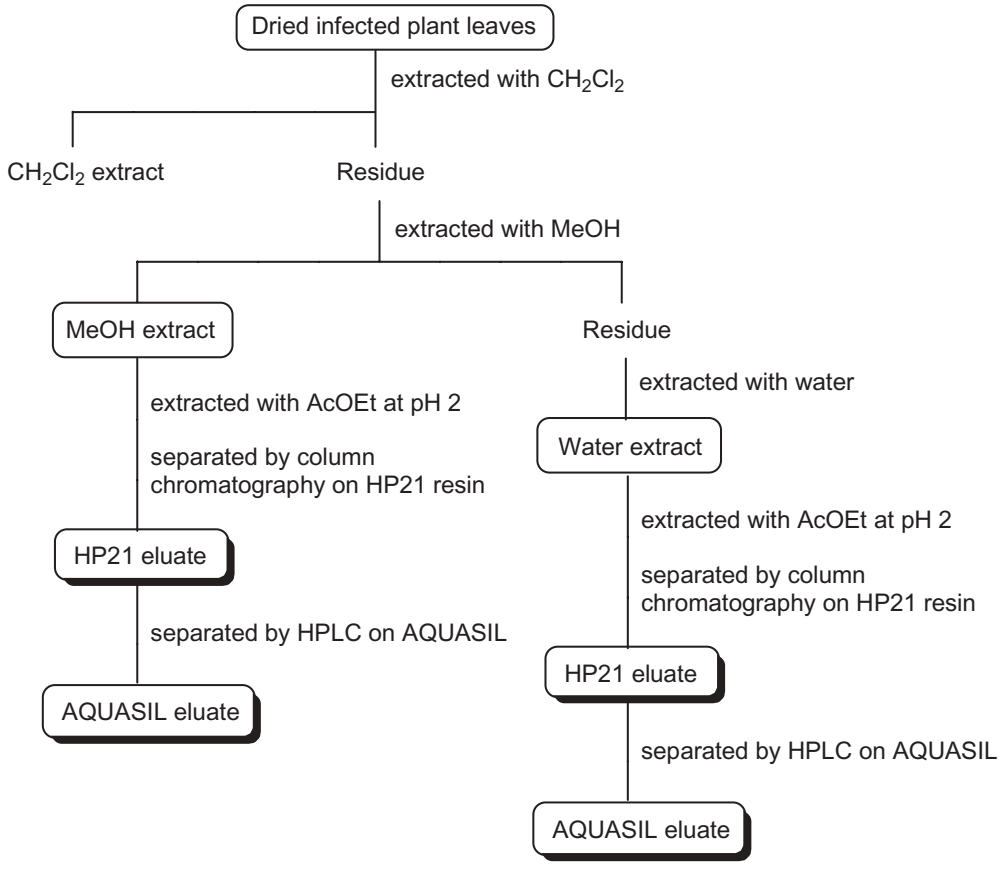

Fig. 1. Separation of active fractions in rust-infected plant leaves

on an HP21 resin column (DIAION) (60 ml resin volume in water; 20 mm inside-column diameter) by elution with 240 ml water, 60 ml 10% MeOH, and then 90 ml MeOH (30 ml/fraction) at a flow rate of 3 ml/min to give 11 fractions of HP21 eluate (fractions 1–10 and MeOH fraction). All fractions that showed teliospore-inducing activity were combined, concentrated in vacuo, and then further separated by high performance liquid chromatography (HPLC) on a Senshu Pak AQUASIL SS-5251 (20 × 250 mm), eluted with a MeOH–water gradient solvent system (initially, 95% MeOH for 10 min; then increasing to 5% MeOH in 45 min; then remaining for 5 min at a flow rate of 8 ml/min) to obtain 30 fractions of AQUASIL eluate (2 min/fraction, fractions 1–30) (see Tables 3, 4).

Bioassays for teliospore-forming activity were performed as previously described with slight modification (Hosoe et al. 2001). Briefly, 5 days after inoculation with urediniospores of wheat leaf rust, cotyledons of wheat cultivar Norin no. 61 with flecking were cut into 4-cm-long segments and placed in a 5-ml glass vial with a 2-ml sample solution. The sample solution was prepared by drying 2 ml each of HP-21 or AQUASIL fraction eluate in a glass vial with a blast dryer at 45°C overnight. Samples were then bioassayed after resuspension in 2 ml distilled water. Bioassay results were evaluated as described previously (Hosoe et al. 2001).

The results of bioassay for the telia-inducing activity are shown in Table 2 for HP21 eluates of MeOH and water extracts and in Tables 3 and 4 for AQUASIL eluates of MeOH and water extracts. Some of the HP21 fraction eluates of MeOH and water extracts of all the rust-infected leaves induced teliospore production of *P. recondita* f. sp. *tritici,* except for no. 6 MeOH extracts and no. 17 water extracts (see Table 2). Some AQUASIL fraction eluates,

Table 2. Teliospore-inducing activity of HP-21 eluate from MeOH and water extracts

No.	Sample no.	Teliospore-inducing activity	
		MeOH extract	Water extract
15	1	○	○
13	2	○	○
14	3	○	○
16	4	○	○
17	5	○	○
12	6	×	○
11	7	○	○
18	8	○	○
19	9	○	○
6	10	○	○
7	11	○	○
3	12	○	○
4	13	○	○
5	14	○	○
2	15	○	○
1	16	○	○
10	17	○	×
9	18	○	○
8	19	○	○

○, sample showed activity; ×, sample did not show activity

Table 3. Teliospore-inducing activity of AQUASIL eluate from MeOH extracts

No.	Sample no.	Fraction no.																													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
13	4	–	–	–	–	+	+++	+++	+	+	+	+	+	+	+	+	–	–	+	–	+	–	–	–	–	–	–	–	–	–	–
14	5	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	D	–	D	D	D	D	–	–	D	–
11	7	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
19	8	+	–	D	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	++	++	+	+	++	+++	+	+	+	D	+	+
18	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	D	D	D	D	D	D	D	D	D	D	D
7	11	–	+	–	–	–	–	+	+	+	+	+	+++	+	+	+	+	+	–	–	–	+	–	+	–	–	–	–	–	–	+
6	12	–	–	–	–	–	–	–	–	–	–	+	–	+++	+++	++	+	+	+	–	–	–	–	–	–	–	–	+	–	–	–
2	13	+	–	–	D	–	–	+	+	+	+++	+	+++	+++	+++	+++	+++	+++	+	+	+	–	–	–	+	+	D	D	–	–	–
3	14	–	–	+	–	–	–	–	–	+	++	++	+++	+++	++	++	++	+++	+	+	D	D	+	+	D	–	D	D	–	–	+
1	15	+	+	–	–	–	–	+	++	+	++	+++	+++	+++	+	+++	+++	++	+	+	+	–	+	–	–	+	–	–	–	–	–
4	16	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	–	–	+	–	–	–	–	–	D	–	D	D	D	D
5	17	+	+	+	–	+	–	+	–	+	+	+	–	+	+	+	+	++	+	+	–	–	–	–	+	–	–	–	+	–	+
8	18	+	–	–	–	–	–	–	++	–	–	–	+	–	+	+++	+++	+	+	+	+	–	–	–	–	–	+	–	–	F	–
9	19	–	–	D	–	–	+	–	+	–	–	+	+	+++	+++	++	+	+	++	–	–	D	–	–	–	–	D	–	–	–	D

Samples nos. 1, 2, 3, and 10 did not clearly show the activity in any fraction
+++, strong (telia completely encircling a green island); ++, medium (telia partially encircling a green island); +, weak (a few telia in or around a green island); –, no telia formation; D, death of wheat leaf; F, growing other fungus

Table 4. Teliospore-inducing activity of AQUASIL eluate from water extracts

No.	Sample No.	Fraction no.																													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
16	1	+	–	–	–	–	–	–	+	–	D	–	–	–	+++	+	–	+	–	–	–	–	–	–	+	–	–	+	–	+	–
15	2	–	–	+	–	+	+	+	++	++	+++	+++	+++	+++	+++	+	+++	++	+	–	+	–	–	–	++	+	–	–	–	–	–
12	3	–	–	–	–	–	–	+	–	–	–	–	+	++	+++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
13	4	–	–	–	–	+++	+++	+++	+	+	+++	+	+	+	–	+	+	+	+	+	–	–	–	–	–	–	–	–	+	–	–
14	5	–	–	–	D	–	–	–	+	–	–	–	–	++	+++	+++	++	+++	–	–	+	–	–	–	–	–	–	–	–	–	–
10	6	+	–	–	D	–	–	+	+	–	–	+	+	+	+++	++	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
11	7	–	+	–	–	–	+	–	–	–	–	–	+	+	+++	D	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
19	8	–	–	–	F	+	++	+++	+++	+++	+++	++	+	+++	+++	+++	+++	++	++	++	+	+	+	+	+	–	–	–	–	–	–
18	9	–	–	+	D	–	–	+	–	–	–	+	+	–	+	+++	+	+	–	+	–	–	–	–	–	–	–	–	–	–	–
17	10	–	–	–	–	–	–	–	–	–	–	–	+	+	+	+	–	–	–	–	D	D	D	D	D	D	D	D	D	D	D
7	11	–	–	–	D	–	+	–	–	+	+	+	++	++	++	+	+	+	–	–	+	–	+	–	–	–	+	–	–	–	–
6	12	–	–	+++	–	+	+	+	++	++	+	+	++	+++	+	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
2	13	–	–	–	D	–	+	D	+	–	+	+	++	+++	+++	+	++	+++	+	+	–	–	–	–	–	–	–	–	–	–	–
3	14	+	–	–	+	++	+	–	+	+	+	–	+	++	+++	+++	+++	+++	–	+	+	+	–	–	–	–	–	–	–	–	–
1	15	–	–	–	–	–	–	+	–	+	+	–	+	+++	+++	+	–	+	–	+	–	–	–	+	–	–	–	+	+	+	–
4	16	+	–	+	–	+	–	–	–	–	+	+	+++	++	+	+++	+	+	–	++	+	–	+	+	–	+	–	–	+	+	+
8	18	+	–	–	–	–	–	–	–	–	–	+	+	+	++	+++	++	+	+	++	+	+	–	–	–	–	D	–	–	–	–	–
9	19	–	–	–	+	+	–	+	–	–	++	+++	+++	+++	+++	+++	+	++	+	+	–	–	–	–	–	–	–	–	–	–	–	–

+++, strong (telia completely encircling a green island); ++, medium (telia partially encircling a green island); +, weak (a few telia in or around a green island); –, no telia formation; D, death of wheat leaf; F, growing other fungus

which were further purified from the active HP21 eluates, induced teliospore production of *P. recondita* f. sp. *tritici*, except for those from MeOH extracts of nos. 1, 2, 3, and 10 (see Tables 3, 4).

We previously reported that substances inducing teliospore production of wheat leaf rust (*P. recondita* f. sp. *tritici*) were present in rust-infected wheat leaves with abundant telia at harvest (Hosoe et al. 2001). In the present study, we investigated the teliospore-inducing activity for wheat leaf rust of the leaves infected with rust fungi belonging to 18 species in 6 families: Pucciniaceae, Melampsoraceae, Coleosporiaceae, Pileolariaceae, Phragmidiaceae, and Phakopsoraceae. The teliospore-inducing activity was found in almost all MeOH and/or water extracts of rust-infected leaves with teliospores (see Table 2). Bioassay results after separation by HPLC on the AQUASIL column (AQASIL eluate) showed that telia-inducing active fractions derived from rust-infected leaves did not always appear as a single peak. Thus, we presumed that each infected leaf might contain several active compounds (see Tables 3, 4).

Because of difficulty in collecting host plants in the field that were certain to be not infected, extracts from uninfected host plants were not tested here. In a previous study, we reported extracts from wheat leaves with abundant telia showed significantly stronger activity than those from uninfected wheat leaves (Hosoe et al. 2001). We concluded that the rust-infected host plants or rust fungi themselves produced the substances.

Teliospores of rust fungi are generally produced on host plants when the plants approach maturity (Takahashi et al. 1965; Agrios 1997) or suffer from unfavorable rust conditions (Warter 1928; Biali et al. 1972; Yeh et al. 1981; Völker and Boyle 1994). Thus, in previous studies, the teliospore-inducing factors were isolated from wheat leaves with teliospores of wheat leaf rust just before harvest time (Hosoe et al. 2001). In this study, the extract of *Chrysanthemum morifolium* Ramat. infected with *P. horiana* Henn. (no. 11) had teliospore-inducing activity for wheat leaf rust. This rust is a microcyclic species, which has only basidiospores and teliospores in the life cycle. Thus, teliospores were produced even on young host leaves. *Coleosporium* species produced teliospores that germinate without dormancy relatively earlier than species for which teliospores germinate after overwintering (Hiratsuka et al. 1992; Cummins and Hiratsuka 2003). Extracts from samples nos. 1 and 2 (*Coleopsorium* spp.) also showed teliospore-inducing activity. These results indicated that these teliospore-inducing factors are unrelated to the host plant senescence and strongly suggested that the rust fungus, rather than the host plant, produced the substances.

Furthermore, these results showed that the teliospore-inducing factors do not significantly stimulate host plant senescence. If the substances do not affect host plant physiology, they may be commercially useful for controlling rust epidemics by urediniospores without damaging the host plants, although the host plant effect remains to be examined.

Here we detected teliospore-inducing activity in almost all rust-infected leaves by bioassay using wheat leaf rust. However, similarity between teliospore-inducing factors acquired from many kinds of rust-infected leaves remains uncertain. Further studies are required to discover teliospore-inducing factors that may be produced by other species of rust fungi.

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