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Morphological and physiological comparisons of *Helicobasidium mompa* and *H. purpureum*

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Morphological and physiological comparisons were made of seven *Helicobasidium mompa* isolates and four *H. purpureum* isolates. Colonies of the *H. mompa* isolates were thin, dense, or hard and dense, and most were pale brown to brown or dark brown, while that of isolate 344c was pinkish. Colonies of *H. purpureum* isolates were hard and dense, and their colonies were dark brown. Diameters of hyphae were similar for *H. mompa* and *H. purpureum*. Dimensions of conidia and morphology of conidiophores of *H. mompa* isolate 344c were close to those of *H. purpureum* reported previously. *H. mompa* isolates grew well at 23°C, 25°C or 27°C, while all isolates of *H. purpureum* grew well at 23°C. Growth rates of *H. purpureum* isolates was almost the same as those of *H. mompa* isolates with slow growth. Polygaracturonase activity at pH 3 was variable among the isolates for both *H. mompa* and *H. purpureum*. Itaconic acid was produced abundantly by three isolates of *H. mompa* but not produced by isolate AH130, whereas all isolates of *H. purpureum* produced a small amount of itaconic acid.

Key Words——*Helicobasidium mompa*; *Helicobasidium purpureum*; morphological and physiological comparisons.

Introduction

Violet root rot caused by *Helicobasidium mompa* (Tanaka, 1891) is a serious disease on apple, mulberry, asparagus, and so on. The violet root rot in Europe and America was first described by Tulasne (1865) and Tulasne and Tulasne (1871, 1872), and Patouillard (1885) ascribed the pathogen to the genus *Helicobasidium*, adopting the species name of *H. purpureum*. Later, Buddin and Wakefield (1927, 1929) indicated that *H. purpureum* **(Tul.) Pat.** was the perfect state of *Rhizoctonia crocorum* **(Pers.) DC.**, which was described by Duggar (1915).

The violet root rot fungus in Japan was reported to be distinct from *H. purpureum* in the color of the pileus and the number of basidiospores on a basidium (Tanaka, 1891), the color of germ tubes or primary hyphae, the presence or absence of conidia of *Tuberculina* type, symptoms, pathogenicity and parasitism (Ito, 1949). However, Suzuki et al. (1957) indicated that it was difficult to differentiate these two species by the cultural and morphological characteristics then known. Takai (1967) reported that eight strains of *H. mompa* produced helicobasidin but one strain of *H. purpureum* did not. Buddin and Wakefield (1927) observed conidia of *Tuberculina* type in culture of *H. purpureum*, and recently Arai et al. (1987) discovered isolates of *H. mompa* which form conidia in culture from apple and mulberry trees. Because *H. mompa* and *H. purpureum* appear to resemble each other closely, comparison of more *H. mompa* and *H. purpureum* isolates is needed.

The aim of the experiments described here is to make morphological and physiological comparisons of seven *H. mompa* isolates and four *H. purpureum* isolates. A preliminary report of this work has been published (Sayama et al., 1991).

Materials and Methods

Fungus Seven isolates of *H. mompa* and four isolates of *H. purpureum* (Import permit No. Yokoshoku 1667) were used (Table 1). These isolates were maintained on potato dextrose agar (PDA) at 25°C and transferred to fresh PDA every two weeks. Only *H. mompa* isolate 344c formed conidia on PDA.

Morphological comparison Two-week cultures on PDA were used for morphological comparison.

Growth on PDA at different temperatures Five-mm agar discs from Petri plate cultures of seven *H. mompa* and four *H. purpureum* isolates were placed on fresh PDA in plates and incubated for ten days at different temperatures. The diameter of each colony was measured to determine the effect of temperature on fungal growth.

Measurements of polygaracturonase (PG) For PG assay, 100 ml portions of a modified Richards' medium (citrus pectin 12.5 g, peptone 10 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, NH_4NO_3 0.5 g, KH_2PO_4 0.5 g, 2% $FeCl_2$ 0.3 ml, D. W. 1 l, pH 4) were dispensed into 300 ml flasks and autoclaved at 120°C for 20 min. Each flask was inoculated with three 1 cm plugs cut from PDA culture. After two months, cultures were vacuum-filtered through What-

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man No. 1 filter paper, and mycelial mats were oven-dried at 80°C and weighed. The filtrate was brought to 50% of saturation with ammonium sulfate and centrifuged at 10,000 r.p.m., for 20 min. The pellet was discarded, and supernatant was brought to 95% of saturation with ammonium sulfate, then centrifuged at 10,000 r.p.m., for 20 min. The pellet was suspended in distilled water and centrifuged at 10,000 r.p.m., for 20 min to remove undissolved material. The supernatant was dialyzed against several hundred volumes of distilled water at 4°C. Protein concentration in the resulting solution was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard, then adjusted to 500 μg/ml. Samples were stored under toluene at 4°C until assay. PG activity based on change in the viscosity of reaction mixture at 38°C was determined using Ostwald viscometers. Reaction mixtures contained 10 ml of 0.5% pectin solution, 2 ml of 0.1 M citrate and 0.2 M phosphate buffer (pH 3) and 1 ml of enzyme solution, since the enzyme was the most ac-

Table 1. Fungus isolates used.

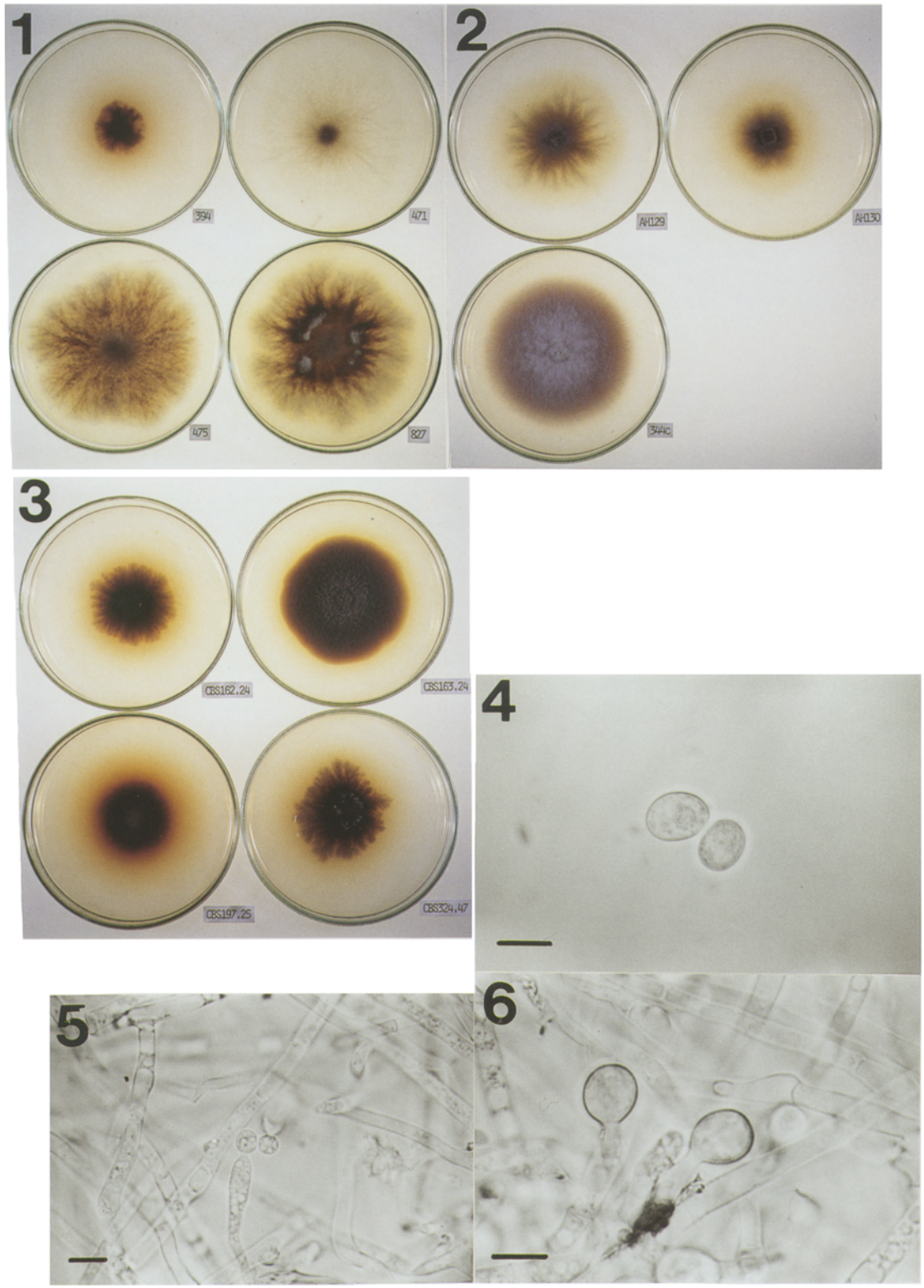
Isolate	Source	Locality	Date of collection
Helicobasidium mompa			
344c*	*Morus bombycis*	Aomori	Jun. 1983
394	*Daucus Carota* var. *sativa*	Aomori	Jun. 1984
471	*Morus bombycis*	Aomori	Apr. 1984
475	*Malus pumila* var. *dulcissima*	Akita	Sep. 1983
827	*Malus pumila* var. *dulcissima*	Aomori	unknown
AH129**	*Malus pumila* var. *dulcissima*	Aomori	Jul. 1985
AH130**	*Malus pumila* var. *dulcissima*	Aomori	Jul. 1985
H. purpureum			
CBS162.24	*Solanum tuberosum*	unknown	unknown
CBS163.24	*Beta vulgaris*	unknown	unknown
CBS197.25	*Trifolium* sp.	unknown	unknown
CBS324.47	unknown	unknown	unknown

* Isolate forming conidia in culture.
** Single conidium isolate.

Table 2. Cultural characteristics of isolates of *Helicobasidium mompa* and *H. purpureum*.

Isolate	Colony character (texture and color)
H. mompa	
344c*	dense; pink
394	hard, dense; dark brown
471	thin; pale brown
475	dense; brown
827	dense; brown
AH129**	thin; brown
AH130**	hard, dense; brown
H. purpureum	
CBS162.24	hard, dense; dark brown
CBS163.24	hard, dense; dark brown
CBS197.25	hard, dense; dark brown
CBS324.47	hard, dense; dark brown

* Isolate forming conidia in culture.
** Single conidium isolate.



Figs. 1-3. Colonies of *Helicobasidium mompa* and *H. purpureum* on PDA after two weeks.
1. *H. mompa* isolate 394, 471, 475 and 827.
2. *H. mompa* isolate AH129, AH130 and 344c.
3. *H. purpureum* isolate CBS162.24, CBS163.24, CBS197.25 and CBS324.47.

Figs. 4-6. Conidia (4) and conidiophores (5, 6) of *H. mompa* isolate 344c (scale: 10 μm).

tive under acidic conditions (Yamazaki, 1957). Enzyme activity was expressed as percentage decrease in viscosity (D), calculated with the formula: D=(100(Ts-Tt)/Ts-Tw), where Ts is the flow time of the substrate control, Tt is the flow time of the test, and Tw is the flow time of water. Results were recorded as percentage reduction in viscosity/120 min.

Itaconic acid productivity Potato sucrose broth was used for testing itaconic acid productivity. Thirty-ml portions of the medium were dispensed into 100-ml flasks and autoclaved at 120°C for 20 min. Each flask was inoculated with five 5-mm plugs cut from PDA culture. After two months, the contents of each flask were filtered. Dry weight of mycelium was determined after drying at 80°C overnight. Filtrate pH was measured, and itaconic acid content was determined by titration with 0.1 N sodium thiosulfate solution ($Na_2S_2O_3$), following the procedure outlined by Friedkin (1945). To standardize this method, a linear relationship was established by titrating known quantities of certified itaconic acid (Wako) with 0.1 N $Na_2S_2O_3$. Itaconic acid productivity was expressed as the total weight (mg) of itaconic acid per gram dry weight mycelium.

Results

Morphological characteristics Morphological characteristics of the isolates of *Helicobasidium mompa* and *H.*

Table 3. Diameter of hyphae of *Helicobasidium mompa* and *H. purpureum* on potato dextrose agar.

Isolate	Diameter of hyphae (μm)	Mean
H. mompa		
344c*	2.0-6.7	4.8
394	2.7-5.3	3.7
471	2.0-4.7	3.6
475	2.7-5.3	4.0
827	2.7-6.0	4.4
AH129**	2.0-5.3	3.8
AH130**	2.0-4.7	3.4
H. purpureum		
CBS162.24	2.7-5.3	4.4
CBS163.24	2.7-6.7	3.7
CBS197.25	2.7-4.7	3.8
CBS324.47	2.0-6.0	3.9

* Isolate forming conidia in culture.
** Single conidium isolate.

Table 4. Effect of temperatures on mycelial growth of *Helicobasidium mompa* and *H. purpureum*.

Isolate	10°C	15°C	20°C	23°C	25°C	27°C	30°C	35°C
H. mompa								
344c*	7.5***	16.5	27.3	41.1	43.5	45.5	11.8	0
394	8.5	15.8	24.5	29.2	27.8	24.9	18.3	0
471	8.0	21.8	42.9	52.6	55.1	50.0	24.0	0
475	7.9	22.5	38.1	48.6	44.8	41.3	35.6	0
827	8.5	25.8	44.6	51.8	49.9	45.1	27.6	0
AH129**	5.0	9.4	26.8	33.6	38.8	31.5	19.9	0
AH130**	5.9	13.3	26.4	33.5	34.0	37.1	23.4	0
H. purpureum								
CBS162.24	7.6	13.3	20.0	23.3	21.1	18.5	13.8	0
CBS163.24	11.1	22.5	34.1	37.0	36.8	29.8	6.6	0
CBS197.25	9.3	20.6	26.1	27.5	24.3	20.4	8.8	0
CBS324.47	7.1	17.1	29.6	36.9	34.9	21.3	9.8	0

* Isolate forming conidia in culture.
** Single conidium isolate.
*** mm/ten days.

purpureum differed from one isolate to another (Table 2 and Figs. 1-3). Colonies of some *H. mompa* isolates were thin, but others were dense or hard and dense. Those of *H. purpureum* isolates were hard and dense. Colonies of most *H. mompa* isolates were pale brown, brown or dark brown, while isolate 344c was pinkish. Colonies of the four *H. purpureum* isolates were dark brown. Hyphae of the *H. mompa* isolates had almost the same diameter as those of *H. purpureum* (Table 3). Isolate 344c of *H. mompa* formed globose or ovoid conidia (5.5-10.9×4.1-8.2 μm) on "*Tuberculina*" type conidiophores (Figs. 4-6).

Growth on PDA at different temperatures Growth of *H. mompa* was optimum at 23°C, 25°C or 27°C depending on the isolate. The optimum temperature for growth of all *H. purpureum* isolates was 23°C (Table 4).

PG activity PG activities of *H. mompa* and *H. purpureum* varied depending on the isolate. Isolates 344c, 475 and 827 of *H. mompa* showed strong activities, while isolates 394 and AH130 showed weak ones. Isolate CBS 324.47 of *H. purpureum* showed strong activity, but other *H. purpureum* isolates showed weak activities (Table 5).

Itaconic acid productivity Itaconic acid productivities of *H. mompa* isolates 475 and 827 were all high, while isolate AH130 did not produce itaconic acid. All four isolates of *H. purpureum* produced a small amount of itaconic acid (Table 6).

Table 5. Polygaracturonase activity of *Helicobasidium mompa* and *H. purpureum*.

Isolate	Total protein (mg/g dry fungus)	Viscosity (%/0.5 mg protein)
H. mompa		
344c*	13.96	92.29
394	11.77	7.68
471	25.98	68.67
475	51.71	94.07
827	16.44	99.35
AH129**	32.29	64.43
AH130**	17.43	3.03
H. purpureum		
CBS162.24	62.67	36.28
CBS163.24	3.69	17.25
CBS197.25	19.84	2.07
CBS324.47	14.99	79.20

* Isolate forming conidia in culture.
** Single conidium isolate.

Table 6. Itaconic acid productivity of *Helicobasidium mompa* and *H. purpureum*.

Isolate	pH of filtrate	Itaconic acid productivity (mg/g dried mycelia)
H. mompa		
344c*	7.30	134.2
394	4.90	96.2
471	3.90	99.3
475	2.95	2103.5
827	3.10	1676.2
AH129**	3.50	853.2
AH130**	5.30	0.0
H. purpureum		
CBS162.24	4.55	605.7
CBS163.24	4.85	386.0
CBS197.25	4.75	207.9
CBS324.47	4.30	225.5
Control	6.30	0.0

* Isolate forming conidia in culture.
** Single conidium isolate.

Discussion

Cultural characteristics of *H. mompa* differed from isolate to isolate in texture and color. Colonies of four *H. purpureum* isolates were hard and dense, and dark brown in color. Colonies of *H. purpureum* isolates on the whole resembled that of *H. mompa* isolate 394. Diameters of hyphae of *H. mompa* isolates were similar to those of *H. purpureum*. An important difference between *H. mompa* and *H. purpureum* has been stated to be the lack of a conidial stage in *H. mompa* (Ito, 1949., Suzuki et al., 1957). However, recently Arai et al. (1987) reported the imperfect stage of *H. mompa*. The morphology of conidiophores and conidia of *H. mompa* isolate 344c closely resembled those of *H. purpureum* reported by Valder (1958), though the description by Buddin and Wakefield (1927) was slightly different. Four isolates of *H. purpureum* did not form conidia in vitro.

The optimum temperature for growth of *H. mompa* isolates was between 23°C and 27°C, as has been described by Ieki (1967). That of the four *H. purpureum* isolates was 23°C, though Buddin and Wakefield (1924) and Whitney (1954) stated that the optimum temperature of *H. purpureum* was about 25°C. Growth rates of the four *H. purpureum* isolates resembled those of the *H. mompa* isolates 394, AH129 and AH130, with slow growth.

During the infection process of *H. mompa*, PG and itaconic acid play important roles (Yamazaki, 1957; Araki et al., 1957; Kubomura et al., 1972). PG activities of both *H. mompa* and *H. purpureum* isolates varied among the isolates. Furthermore, isolate AH130 of *H. mompa* isolates did not produce itaconic acid.

As shown above, *H. mompa* and *H. purpureum* resemble each other closely and we need more detailed studies to clarify whether they are truly separate species or not.

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