RED PIGMENT FORMATION BY INTERACTION OF MOLDS

PART 4 INTERACTION BETWEEN *P. VERRUCULOSUM* AND VARIOUS MOLDS

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It was reported in previous papers (1-3) that some red pigments were produced by interaction between *Penicillium verruculosum* and some strains or species of the genera *Penicillium* and *Trichoderma*. It was inferred that the pigments are derived from their colorless precursor(s) formed by *P. verruculosum*, which are later converted into the colored material by the action of some other molds. In order to confirm the validity of this inference, further investigations were carried out on the possibility of red pigment formation by several strains of *P. verruculosum* grown in combination with various strains belonging to the genera *Aspergillus*, *Rhizopus*, *Mucor* as well as those belonging to the classes *Deuteromycetes* (Fungi Imperfecti) and *Basidiomycetes*.

EXPERIMENTAL METHODS

1) *Microorganisms*: The strains tested as possible producers of precursors were strain L 9 isolated by the authors and strains I. A. M. 7058, 7064, 7067, 7073 and 7079 of *P. verruculosum* obtained from the collection at the Institute of Applied Microbiology, University of Tokyo. As secondary molds which are supposed to convert the colorless precursor(s) into the red pigments, tests were made with some representative species of the genera *Aspergillus* (Table 1), *Rhizopus* (Table 2) and *Mucor* (Table 2), obtained from the same collection, and some representative species of the classes *Deuteromycetes* (Tables 3 and 4) and *Basidiomycetes* (Table 5) which were provided generously by the Institute of Fermentation, Ministry of International Trade and Industry.

2) *Pigment formation*: The culture plates were prepared with glucose-peptone agar containing 4.0% glucose and 1.0% Difco-peptone, (pH 6.0). Spores of each strain of *P. verruculosum* and spores or mycelial fraction of each strain of the secondary molds were inoculated at a distance of about 3 cm on the same plate. They were incubated at 30° for 5 to 7 day, and the formation of the red pigments was observed during the course of incubation

Table 1. Red pigment formation in co-biontic cultures of several strains of *P. verruculosum* with some species of the genus *Aspergillus*.

<i>P. verruculosum</i>	Diameter of colony, mm	Sporulation	I.A.M. 7058	I.A.M. 7064	I.A.M. 7067	I.A.M. 7073	I.A.M. 7079	L 9
<i>Aspergillus</i>								
<i>A. clavatus</i>	44	(-)	(-)	(-)	(-)	$\frac{\text{##}}{\text{#}}$	(-)	(-)
<i>A. glaucus</i>	22	(-)	(-)	(-)	(-)	$\frac{\text{##}}{+}$	(-)	(-)
<i>A. fumigatus</i>	80	(-)	(-)	$\frac{(-)}{\text{#}}$	(-)	$\frac{\text{##}}{\text{##}}$	(-)	(-)
<i>A. nidulans</i>	84	(-)	(-)	$\frac{(-)}{\text{#}}$	(-)	$\frac{+}{\text{##}}$	(-)	(-)
<i>A. flavipes</i>	86	+	(-)	$\frac{\text{#}}{\text{#}}$	$\frac{\text{##}}{\text{#}}$	$\frac{\text{##}}{\text{##}}$	(-)	$\frac{(-)}{\text{##}^*}$
<i>A. versicolor</i>	10	##	(-)	(-)	$\frac{\text{#}}{+}$	$\frac{\text{##}}{+}$	$\frac{+^*}{\text{#}}$	$\frac{(\pm)}{+}$
<i>A. niger</i>	100	##	(-)	$\frac{+}{(-)}$	$\frac{+}{\text{#}}$	$\frac{\text{##}}{\text{#}}$	$\frac{(-)}{+}$	$\frac{\text{#}}{\text{#}}$
<i>A. tamaraii</i>	84	+	$\frac{(-)}{\text{##}^*}$	$\frac{(-)}{\text{##}^*}$	$\frac{\text{#}}{\text{##}}$	$\frac{\text{##}}{\text{##}^*}$	$\frac{(-)}{\text{##}^*}$	$\frac{(-)}{\text{##}^*}$
<i>A. flavusoryzae</i>	90	##	(-)	$\frac{+}{\text{#}}$	$\frac{(-)}{\text{#}}$	$\frac{\text{##}}{\text{##}}$	(-)	(-)
<i>A. ochraceus</i>	40	+	(-)	(-)	(-)	$\frac{\text{#}}{\text{#}}$	(-)	(-)

* : Yellow pigment.

** : Colonies of *A. niger* overlapped on the colonies of *P. verruculosum*. The marks above and below the bar represents, respectively the degree of pigment formation in *P. verruculosum* and in *Aspergillus*.

EXPERIMENTAL RESULTS

1) *Pigment formation under interaction with Aspergillus*: The results obtained with combined cultures of several strains of *P. verruculosum* with 10 representative species of the genus *Aspergillus* are preserbred in Table 1. Strain I. A. M. 7073 produced much red pigments, while I. A. M. 7058 and 7079 did not produce the pigments with all the *Aspergilli* tested. In the combinations of some strains of *P. verruculosum* with *A. flavipes*, *A. tamaraii* or *A. versicolor*, yellow pigments were produced instead of the red ones. These yellow pigments were generally found along the side of *Aspergillus*

Table 2. Red pigment formation by interaction of several strains of *P. verruculosum* with some species of the genus *Rhizopus* and *Mucor*.

<i>P. verruculosum</i> <i>Rhizopus,</i> <i>Mucor</i>	Diameter of colony,* mm	Sporulation**	I.A.M. 7058	I.A.M. 7064	I.A.M. 7069	I.A.M. 7073	I.A.M. 7079	L 9
<i>R. nigricans</i>	70>	≡	(-)	≡	(-)	(-)	(-)	(-)
<i>R. javanicus</i>	70>	≡	(-)	(-)	(-)	(-)	(-)	(-)
<i>R. chinensis</i>	70>	≡	(-)	+	(-)	(-)	(-)	(-)
<i>R. arrhizus</i>	70>	(-)	(-)	+	(-)	(-)	(-)	(-)
<i>R. salebrosus</i>	70>	≡	(-)	+	+	(-)	(-)	(-)
<i>R. candidus</i>	70>	≡	(-)	≡	+	(-)	(-)	(-)
<i>M. spinescens</i>	70>	≡	(-)	≡	(-)	(-)	(-)	(-)
<i>M. racemosus</i>	32	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>M. plumbeus</i>	70>	≡	(-)	≡	+	(-)	(-)	(-)

* : Diameter of colony of *Rhizopus* or *Mucor*.

** : Sporulation of *Rhizopus* or *Mucor*.

colonies except in the combination of *P. verruculosum* with *A. versicolor*. Mycelia of *P. verruculosum* overlapped with those of *A. niger*, but not with those of the other species of *Aspergillus*. Non-overlapping would be due to some antibiotic substances which were produced and exerted from mycelia of the strains tested. Fig. 1 shows some examples of the red pigment formation occurring between the colonies of *P. verruculosum* and *Aspergillus*.

2) *Pigment formation under interaction with Rhizopus and Mucor*: As shown in Table 2 only two strains of *P. verruculosum*, i.e. I.A.M. 7064 and 7067, produced the red pigments when grown together with *Rhizopus* or *Mucor*. All strains of *verruculosum* formed no pigment when in contact with *R. javanicus* and *racemosus*. Negative results were also obtained when strain L 9 of *P. verruculosum* was co-cultured with each strain of *Rhizopus* and *Mucor* examined.

3) *Pigment formation under interaction with Deuteromycetes*: Formation of the red pigments occasionally occurred when *P. verruculosum* was grown together with some strains of *Deuteromycetes* (Tables 3 and 4). Especially, strain I.A.M. 7073 of *P. verruculosum* formed a considerably large amount of the red pigments when grown in combination with various strains of

Table 3. Red pigment formation by interaction of several strains of *P. verruculosum* with representative species of *Deuteromycetes* (I).

<i>P. verruculosum</i>	Diameter of colony mm	Colony reverse	I.A.M. 7058	I.A.M. 7064	I.A.M. 7067	I.A.M. 7073	I.A.M. 7079	L 9
<i>Deutero mycetes</i> (Fungi Imperfecti)								
<i>Moniliales</i>								
<i>Moniliaceae</i>								
<i>Acremonium potronii</i>	15	—	(—)	(—)	(—)	(—)	(—)	(—)
<i>Acrostalagmus albus</i>	38	White	(—)	+/+	(—)	+/#	(—)	(—)
<i>Beauveria bassiana</i>	16	Yellow	(—)	(—)	(—)	+/#	(—)	(—)
<i>Botrytis reptans</i>	11	Yellowish green	(—)	(—)	(—)	+/#	(—)	(—)
<i>Candelospora penialloides</i>	26	Yellow	(—)	*#/(—)	(—)	+/#	(—)	(—)
<i>Corethrospis hominis</i>	8	Greenish black	(—)	(—)	(—)	(—)	(—)	(—)
<i>Gliocladium sp.</i>	40	White	(—)	+/+	*+ / +	+ / #	(—)	(—)
<i>Monosporium apiospermum</i>	30	White	(—)	+/ (—)	(—)	(—) / #	(—)	(—)
<i>Mycogone japii</i>	48	Greenish white	(—)	+/ (—)	(—)	+/#	(—)	(—)
<i>Piricularia oryzae</i>	26	White	(—)	+/+	(—)	### / #	(—)	(—)
<i>Spicaria viridans</i>	17	Yellowish brown	(—)	(—)	(—)	(—)	(—)	(—)
<i>Tricothecium roseum</i>	32	White	(—)	Y + / +	(—)	+/#	(—)	Y / # (—)
<i>Tritirachium purpureum</i>	5	Yellow	(—)	(—)	(—)	(—) / #	(—)	(—)
<i>Verticillium niveostratosum</i>	16	White	(—)	(—)	(—)	*+ / #	(—)	(—)
<i>Stilbaceae</i>								
<i>Mycoderma monosum</i>	7	Yellow	(—)	+/ (—)	(—)	+ / +	(—)	(—)
<i>Sporobolomyces roseum</i>	7	Yellowish orange	(—)	(—)	(—)	(—)	(—)	(—)
<i>Microsporium andouini</i>	14	White	(—)	(—)	(—)	(—)	(—)	(—)
<i>Isaria farinosa</i>	31	White	(—)	(—)	(—)	+/#	(—)	(—)
<i>Stysanus medius</i>	40	Grey	(—)	(—)	(—)	+ / +	(—)	(—)
<i>Histoplasma capsulatum</i>	4	White	(—)	### / #	+ / (—)	+/+	(—)	(—)
<i>Parendomyces asteroides</i>	13	White	(—)	(—)	(—)	+/#	(—)	(—)
<i>Pseudomycoderma miso</i>	11	White	(—)	+ / (—)	(—)	### / #	(—)	(—)
<i>Tuberculariaceae</i>								
<i>Epicoccum purpurascens</i>	60	Yellowish orange	(—)	+ / (—)	(—)	+ / #	(—)	(—)
<i>Fusarium oxysporum</i>	80	White	(—)	### / #	+ / (—)	+/+	(—)	(—)

Note: 1) * : Both colonies were overlapped on each other

2) Y : Yellow pigment, (B : brown pigment)

3) The marks below the bars represent pigment formation in *Deuteromycetes* colonies.

4) The marks above the bars represent pigment formation in *Deuteromycetes* colonies.

5) Diameter of colony and reverse side of colony are those of *Deuteromycetes*.

Table 4. Red pigment formation by interaction of several strains of *P. verruculosum* with representative species of Deuteromycetes (II).

<i>P. verruculosum</i>	Diameter of colony, mm	Colony reverse	I.A.M. 7058	I.A.M. 7064	I.A.M. 7067	I.A.M. 7073	I.A.M. 7079	L 9
<i>Deuteromycetes</i> (Fungi Imperfecti)								
<i>Moniliales</i>								
<i>Dermatiaceae</i>								
<i>Cladosporium olivaceum</i>	92	Yellowish white	*(-)/+ Y	*(-)/+ B	B #/+ B	* #/# #	*B #/# #	B #/# #
<i>Altenaria citri</i>	80	Black	(-)	(-)	(-)	* #/# #	(-)	(-)
<i>Cereosporina kikuchi</i>	24	Reddish purple	(-)/+	(-)	(-)	(-)/#	(-)	(-)
<i>Cercospora oryzae</i>	114	Black	*(-)	(-)	(-)	(-)/#	(-)	(-)
<i>Chalara mycederma</i>	6	Yellowish white	(-)	(-)	*(-)	* +/#	(-)	*(-)
<i>Cladosporium herbarum</i>	12	Greenish white	(-)	(-)	*(-)	(-)	*(-)	*(-)
<i>Curvularia tetramera</i>	55	Black	(-)	(-)	(-)	(-)	(-)	(-)
<i>Dematium pullulans</i>	37	Black	*(-)	(-)	(-)	(-)	(-)	(-)
<i>Fumago</i> sp.	6	Black	(-)	(-)	(-)/#	(-)	(-)	(-)
<i>Helicocereas oryzae</i>	2	Black	(-)	(-)	(-)	(-)	(-)	(-)
<i>Helminthosporium avenae</i>	36	White	(-)	(-)	(-)	(-)	(-)	(-)
<i>Hemispora rugosa</i>	16	White	(-)	+ /(-)	(-)	#/# #	(-)	(-)
<i>Humicola grisea</i>	37	Black	(-)	(-)	(-)	+ /#	(-)	(-)
<i>Macrosporium bataticola</i>	12	Black	(-)	+ /+	+ /(-)	(-)/+	(-)	(-)
<i>Nigrospora oryzae</i>	90	Brownish black	(-)	(-)	(-)	+ /#	(-)	(-)
<i>Phialophora verrucosa</i>	10	Black	(-)	(-)	(-)	(-)/#	(-)	(-)
<i>Stachybotrys bicolor</i>	11	White	(-)	+ /(-)	(-)	#/# #	(-)	(-)
<i>Stemphylium botryosum</i>	40	Brownish black	(-)	(-)	(-)	+ /#	(-)	(-)
<i>Thyrospora astragali</i>	35	—	(-)	(-)	(-)	(-)	(-)	(-)
<i>Torulopsis aerea</i>	10	White	(-)	(-)	(-)	#/# #	(-)	(-)
<i>Phomaceae</i>								
<i>Phomaceae</i>								
<i>Ascochyta viciae</i>	5	—	(-)	(-)	(-)	(-)	(-)	(-)
<i>Diplodia natalensis</i>	110	—	(-)	(-)	(-)	* #/+	(-)	(-)
<i>Phomopsis citri</i>	2	—	(-)	(-)	(-)	(-)	(-)	(-)
<i>Melaconiales</i>								
<i>Melaconiaceae</i>								
<i>Gloeosporium laeticolor</i>	32	—	(-)	# /(-)	(-)	+ /#	(-)	(-)
<i>Pestalotia diospyri</i>	26	—	(-)	(-)/#	(-)	#/# #	(-)	(-)
<i>Ustilaginales</i>								
<i>Tilletiaceae</i>								
<i>Tilletiopsis lilacina</i>	6	—	(-)	(-)	(-)	(-)	(-)	(-)

Note: The same as in Table 3.

Table 5. Red pigment formation by interaction of several strains of *P. verruculosum* with representative species of *Basidiomycetes*.

<i>P. verruculosum</i>	Diameter of colony, mm	Colony reverse	I.A.M. 7058	I.A.M. 7064	I.A.M. 7067	I.A.M. 7073	I.A.M. 7079	L 9
<i>Basidiomycetes</i>								
<i>Agricales</i>								
<i>Agricaceae</i>								
<i>Armillaria mellea</i>	3	Yellowish white	(-)	(-)	(-)	###/##	(-)	(-)
<i>Coprinus comatus</i>	7	Yellowish white	(-)	(-)	(-)	(-)	(-)	(-)
<i>Cortinellus edodes</i>	7	Brown	(-)	(-)	(-)	(-)/##	(-)	(-)
<i>Pholiota nameko</i>	4	Yellowish white	(-)	*+/+	+/(-)	##/##	(-)	(-)
<i>Pleurotus serotinus</i>	4	White	*(-)	*+/+	(-)	##/##	(-)	(-)
<i>Psalliota campestris</i>	84	Pale yellow	Y+/+Y	(-)	###/###	(-)/##	(-)/+Y	+/##
<i>Schizophyllum commune</i>	52	Pale yellow	(-)	(-)	*B+/+B	+/##	(-)	*B+/+B
<i>Tricholoma conglobatum</i>	60	White	*(-)	*###/(-)	(-)	##/##	*(-)	*(-)
<i>Polyporaceae</i>								
<i>Coniophora puteana</i>	90	White	(-)	*+/##	+/+	##/##	(-)	(-)
<i>Corticium caeruleum</i>	46	Brownish white	(-)	*##/##	B+/(-)	*##/##	B+/(-)	(-)
<i>Gloeophyllum sepiarium</i>	120	White	(-)	Y##/##	Y##/+B	B+/##	B+/+B	B###/+B
<i>Lenzites lepideus</i>	13	—	(-)	+/##	(-)	+/##	(-)	(-)
<i>Stereum hirsutum</i>	13	White	+/+	(-)/##	(-)	(-)/##	(-)/+	(-)
<i>Trametes albida</i>	60	White	(-)	(-)/##	(-)	*##/##	B+/(-)	B##/(-)
<i>Telephoraceae</i>								
<i>Cryptoporus volvatus</i>	—	—	(-)	(-)	(-)	*##/##	(-)	(-)
<i>Daedalea quercina</i>	44	White	Y+/(-)	##/##	B+/##	+/##	B+/(-)	B+/(-)
<i>Favolus arcularius</i>	60	White	Y+/(-)	*##/(-)	(-)	###/##	*(-)	(-)
<i>Fistulina hepatica</i>	7	White	(-)	(-)	(-)	(-)	(-)	(-)
<i>Fomes fomentarius</i>	2	Yellow	(-)	(-)	(-)	(-)	(-)	(-)
<i>Ganoderma lucidum</i>	44	Pale yellow	(-)	(-)	(-)	Y+/+	(-)	B+/(-)
<i>Merulius confluent</i>	12	White	(-)	(+)/(-)	(-)	+/##	(-)	(-)
<i>Polyporus parustris</i>	66	White	(-)	+/##	+/+	##/##	(-)	*+/+
<i>Poria cocos</i>	39	Brownish black	(-)	(-)	(-)	*##/###	B+/(-)	(-)

Note: The same as in Table 3, except *Basidiomycetes* instead of *Deuteromycetes* in the note (5).

Fig. 1. Red pigment formation by interaction of strains of *P. verruculosum* and those of *Aspergillus*.

P 4: *P. verruculosum* I. A. M. 7073

P 3: *P. verruculosum* I. A. M. 7067

60: *A. niger*

7: *A. tamarii*

Deuteromycetes (more than a half of all strains tested). Some pictures of the pigment formation are presented in Fig. 2. In contrast, strains I. A. M. 7058, 7067, 7079 and L 9 scarcely produced the red pigments under interaction with most strains of *Deuteromycetes*.

4) *Pigment formation under interaction with Basidiomycetes*: Table 5 shows the results obtained when various strains of *P. verruculosum* were co-cultured with different species of *Basidiomycetes*. Some examples of the red pigment formation observed are presented in Fig. 3. In many combinations, strains I.A.M. 7073 and 7064 produced the red pigments remarkably, while strains I.A.M. 7058, 7067, 7079 and L 9 did not do so, although some of them formed yellow and brown pigments. The results in Table 5 show that the formation of yellow and brown pigments is dependent on the strain of *Basidiomycetes* used for the combination and not on that of *P. verruculosum*. The formation was observed only when a strain of *P. verruculosum* was co-cultured with *Gloeophyllum sepiarium*, *Daedalea quercina*, *Ganoderma lucidum*, *Poria cocos* and *Schizophyllum commune*. This observation indicates clearly that the yellow and brown pigments are products

Fig. 2. Red pigment formation by interaction of strains of *P. verruculosum* with those of *Deuteromycetes* (Fungi Imperfecti).

P 2: *P. verruculosum* I. A. M. 7064

P 4: *P. verruculosum* I. A. M. 7073

13: *Candelospora penicilloides* (Moniliaceae)

62: *Hemispora rugosa* (Dermatiaceae)

51: *Pseudomycoderma miso* (Stilbaceae)

primarily formed by *Basidiomycetes*. Whether these yellow and brown pigments have some relation to the red pigments is a question open to further investigations.

SUMMARY

Investigations were made on the phenomenon of red pigment formation occurring in co-biotic cultures of *Penicillium verruculosum* with various other molds belonging to the genera *Aspergillus*, *Rhizopus*, *Mucor* and those

Fig. 3. Red pigment formation by interaction of strains of *P. verruculosum* with those of *Basidiomycetes*.

- P 4: *P. verruculosum* I. A. M. 7073
 P 2: *P. verruculosum* I. A. M. 7064
 57: *Psaliota campestris* (Agricaceae)
 60: *Coniophora puteana* (Polyporaceae)
 70: *Daedalea quercina* (Telephoraceae)

belonging to the classes *Deuteromycetes* and *Basidiomycetes*.

Many species of *Aspergillus* were found to induce the pigment formation in co-cultures with several strains of *P. verruculosum*. An exception was *Aspergillus tamaris* which produced yellow pigment(s) in its mycelia under the same condition. The red pigment formation was seldom observed in the combinations of *P. verruculosum* with some species of *Rhizopus* and *Mucor*. It occurred occasionally in the combinations of various species of *Deuteromycetes* with strain I. A. M. 7073 of *P. verruculosum*, but scarcely with other strains of *P. verruculosum*. Strains I. A. M. 7064 and 7073 of *P. verruculosum* produced the red pigments when grown together with

various molds belonging to *Basidiomycetes*. Some species, especially *Gliophyllum sepium* and *Daedalea gercina*, produced yellow or brown pigment(s) at the contact margin of their colonies with that of *P. verruculosum*.

Thus, the formation of red pigments by *P. verruculosum* in its co-biontic cultures with other molds was found to be a phenomenon of fairly common occurrence.

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