Ascospore Analysis of Kasugamycin Resistance in the Perfect Stage of Pyricularia oryzae

M. Taga, H. Nakagawa, M. Tsuda, and A. Ueyama

Pesticide Research Institute, Kyoto University, Kyoto 606, Japan.

The authors thank M. Aragaki, Universityof Hawaii, for suggestions relative to the preparation of the manuscript, H. Yamamura, Hokko Chemical Industry Co., Ltd, Tokyo, for supplying kasugamycin, and I. Goto, Yamagata University, for sending kasugamycin resistant strains isolated from rice paddy fields.

Accepted for publication 1 November 1977.

ABSTRACT

TAGA, M., H. NAKAGAWA, M. TSUDA, and A. UEYAMA. 1978. Ascospore analysis of kasugamycin resistance in the perfect stage of Pyricularia oryzae. Phytopathology 68: 815-817.

Naturally occurring kasugamycin resistant strains of *Pyricularia oryzae* from rice were crossed with sensitive strains from finger millet. Minimal inhibitory concentrations for growth of progeny isolates were approximately the same value as those for the parent isolates. Using isolates from finger millet, resistant strains selected in vitro were crossed with sensitive strains. In a cross of strains Ta-1-5 × K-7, nine asci and 56 random ascospore isolates were analyzed. Resistance was controlled by a major gene and segregated independently of mating type.

Additional key words: rice blast fungus, antifungal antibiotic, fungal genetics, fungicide resistance.

Kasugamycin is an antibiotic widely used in Japan for the control of rice blast, which is caused by *Pyricularia oryzae* Cavara. In 1971, naturally occuring strains of *P. oryzae* resistant to kasugamycin were found in rice paddy fields where control of rice blast with kasugamycin had failed (3,4).

Kasugamycin resistance in the same organism had been reported earlier by Ohmori (5) who selected resistant strains in vitro and showed that the resistant character was not lost after successive transfers in antibiotic-free medium. Although this stability suggests that resistance to kasugamycin is genetic in nature, neither the mode of inheritance nor the existence of resistant genes has been investigated.

Several investigators (2, 6, 7) have reported the formation of the perfect stage of *P. oryzae* on artificial media by crossing the isolate from rice with the isolate of the opposite mating type from finger millet. This enabled us to study the genetics of resistance to kasugamycin.

In the present paper we report on the genetic nature of kasugamycin resistance in naturally occurring resistant strains of *P. oryzae* and on the mode of inheritance of this resistance in strains selected in vitro.

MATERIALS AND METHODS

Naturally occurring resistant strains were obtained by I. Goto, Yamagata University, from rice paddy fields of Shonai district of Yamagata prefecture in the northeastern part of Japan, where decline in rice blast control first was noticed. Resistant strains in vitro were obtained by a method similar to that of Ohmori (5). A suspension of conidia of the sensitive culture, which had been obtained from finger millet [*Eleusine coracana* (L.) Gaertner], was plated on rice-decoction agar medium containing kasugamycin-HCl at 100 μg/ml (calculated from the purity, 861.0 mcg/mg). This medium was used subsequently as the test medium in all experiments. Small blocks from colonies which appeared on this medium were transferred to fresh test medium. After confirming the resistant character, mycelial blocks were cut from the test medium and used to initiate stock cultures which were cultivated on Misato-Hara medium (1). Strains sensitive to kasugamycin and of opposite mating type to resistant strains were obtained by monoconidial isolation from the diseased grains of finger millet. Each strain used in the cross was maintained in culture tubes on Misato-Hara medium.

Sensitivity of the strains to kasugamycin was evaluated by the plate dilution method. Mycelial disks, 5 mm in diameter, were cut out with a sterilized corkborer from the periphery of 8-day-old mycelial mats growing on Takahashi-A medium (1) at 27 C. Each mycelial disk then was transferred to the test medium in a 9-cm-diameter petri dish and incubated at 27 C. The minimal inhibitory concentrations were determined 2 days after inoculation, and the percentage inhibition of mycelial growth was measured 2, 4, and 6 days after inoculation.

Crosses were attempted between resistant and sensitive strains according to a method previously described (6). Strains to be crossed were inoculated some distance apart on opposite sides of rice straw on Sachs' agar medium in 9-cm-diameter petri dishes and incubated for about 30 days at 24 C.

Some of the perithecia were crushed and monoascospore isolation was done with a micromanipulator. Each ascospore was allowed to germinate and then was transferred to a test tube containing Misato-Hara medium. After incubation for several days, a mycelial disk 4 mm in diameter was cut from the slant and transferred to the test medium.

00032-949X/78/000 138$03.00/0

TABLE 1. Minimal inhibitory concentration (MIC) and percentage inhibition of growth (IG) of ascospore isolates from the cross between the naturally occurring resistant strains, T-4 and T-6, and the sensitive strain K-8 of *Pyricularia oryzae*

Parents and F_1 isolates	Cross T-4 × K-8				Parents and F_1 isolates	Cross T-6 × K-8			
	MIC[x] (μg/ml)	IG at 100 μg/ml[x] (%)		Mating type		MIC[x] (μg/ml)	IG at 100 μg/ml[x] (%)		Mating type
		4 days	6 days				4 days	6 days	
Parents:									
T-4	>200[y]	22[z]	22[z]	A	T-6	>200[y]	9[z]	6[z]	A
K-8	< 20	100	100	a	K-8	< 20	100	100	a
F_1 isolates:									
1	>200	26	45	a	1	>200	7	8	?
2	< 20	100	100	?	2	< 20	100	100	?
3	< 20	100	100	?	3	>200	–	–	?
4	< 20	100	100	?	4	>200	5	5	?
5	< 20	100	100	?					

[x]Rice-decoction agar medium was used for the measurement of MIC and IG.
[y]The MIC was determined 2 days after inoculation.
[z]Mean of two tests.

TABLE 2. Unordered tetrad analysis of asci from the cross of strains Ta-1-5 × K-7 of *Pyricularia oryzae*

Tetrad type	RA	:	Sa	:	Ra	:	SA[a]	Number of asci
Parental ditype	4	:	4	:	0	:	0	1
Nonparental ditype	0	:	0	:	4	:	4	4
Tetratype	2	:	2	:	2	:	2	4
							total	9

[a]Symbols: R = resistant to kasugamycin; S = sensitive to kasugamycin; A = mating type A; and a = mating type a.

Resistance or sensitivity to kasugamycin was determined on the basis of mycelial growth 4 days after inoculation. On the test medium, resistant isolates were clearly distinguishable from sensitive ones. The minimal inhibitory concentration and the percentage inhibition of ascospore isolates both were measured by the above-mentioned method. Mating type of each ascospore isolates was determined with the use of tester strains.

RESULTS AND DISCUSSION

From many crosses between resistant and sensitive strains, the following three crosses produced ascospores: T-4 × K-8; T-6 × K-8; and Ta-1-5 × K-7. Of the resistant strains, T-4 and T-6 were isolated from rice paddy fields, and Ta-1-5 was obtained in vitro. Strains K-7 and K-8 both were sensitive to kasugamycin and compatible with resistant strains.

Crosses T-4 × K-8 and T-6 × K-8 yielded mostly nonviable ascospores. Only five ascospore isolates from the former and four from the latter cross were obtained. As shown in Table 1, both crosses yielded both kasugamycin-sensitive and -resistant progenies. This fact indicates that naturally occurring resistance to kasugamycin is under genetic control, and also suggests that extra-chromosomal factors (i.e., an episome) are not involved. That values for inhibition of mycelial

TABLE 3. Minimal inhibitory concentration (MIC), percentage inhibition of growth (IG), and mating types of eight monoascosporic isolates from an ascus (No. 7) from the cross of strains Ta-1-5 × K-7 of *Pyricularia oryzae*

Parent strains and ascospore number	MIC[w] (μg/ml)	IG[w] (6 days)		Mating type
		20 μg/ml (%)	100 μg/ml (%)	
Parents:				
Ta-1-5	>200[x]	...	9[y]	A
K-7	< 20	...	94	a
Ascospores:				
1[z]	>200	8[y]	16	A
2	>200	2	16	A
3	>200	3	7	a
4	>200	0	6	a
5	< 20	97	97	a
6	< 20	96	96	a
7	< 20	97	100	A
8	< 20	97	100	A

[w]Rice-decoction agar medium was used for the measurement of MIC and IG.
[x]MIC was determined 2 days after inoculation.
[y]Mean of two tests.
[z]Number does not mean the order in the ascus.

TABLE 4. Segregation for kasugamycin resistance and mating type of random ascospore isolates of *Pyricularia oryzae* from the cross of strains Ta-1-5 × K-7

Parental type	Number of random ascospore isolates
RA[a]	14
Sa	17
Recombinant type	
SA	15
Ra	10
total	56

$\chi^2(1:1:1:1)=1.86$ $\chi^2_{.05}=7.82$

[a]Symbols: R = resistant to kasugamycin; S = sensitive to kasugamycin; A = mating type A; and a = mating type a.

growth of ascospore isolates were approximately the same as those of the parental strains is further evidence against polygenic control of resistance. Recombination between resistance and mating type also was observed in the cross, T-4 × K-8. From these results, we believe that naturally occurring resistance to kasugamycin in *P. oryzae* is controlled by a major gene (or genes) located on a chromosome.

When Ta-1-5 was crossed with K-7, nine asci, each of which included eight ascospores, and 56 random ascospore isolates were obtained. Each of the nine asci contained four resistant and four sensitive ascospores (Table 2). Minimal inhibitory concentration and percentage inhibition of growth of eight mono-ascosporic isolates from ascus No.7 were measured (Table 3). These results clearly demonstrate that resistance in strain Ta-1-5 was regulated by one major gene on the chromosome. As shown in Table 2, three kinds of asci (i.e., the parental ditype, the nonparental ditype, and the tetratype) could be recognized by using resistance to kasugamycin and mating type as genetic markers. No linkage between the two loci was evident.

Fifty-six random ascospore isolates were analyzed for resistance to kasugamycin and mating type. The chi-square test also showed that the gene for resistance to kasugamycin and the gene for mating type are located on different chromosomes (Table 4).

Since till now crossing was successful only between Ta-1-5 × K-7, the nature of resistance of other strains selected in vitro is unknown.

LITERATURE CITED

1. ASUYAMA, H., H. MUKO, and N. SUZUKI (eds.). 1962. Laboratory guide for plant pathologists. Nippon Shokubutsu Boeki Kyokai, Tokyo. 843 p. (in Japanese).
2. KATO, H., T. YAMAGUCHI, and N. NISHIHARA. 1976. The perfect state of Pyricularia oryzae Cav. in culture. Ann. Phytopathol. Soc. Japn. 42:507-510.
3. MIURA, H., H. ITO, and S. TAKAHASHI. 1975. Resistant strains of Pyricularia oryzae to kasugamycin as a cause of the diminished fungicidal activity to rice blast. Ann. Phytopathol. Soc. Japn. 41:415-417. (in Japanese with English summary).
4. MIURA, H., M. KATAGIRI, T. YAMAGUCHI, Y. UESUGHI, and H. ITO. 1976. Mode of occurrence of kasugamycin resistant rice blast fungus. Ann. Phytopathol. Soc. Japn. 42:117-123.
5. OHMORI, K. 1967. Studies on characters of Pyricularia oryzae made resistant to kasugamycin. J. Antibiotics A-20:109-114.
6. UEYAMA, A., and M. TSUDA. 1975. Formation of the perfect state in culture of Pyricularia sp. from gramineaceous plants (preliminary report). Trans. Mycol. Soc. Japn. 16:420-422.
7. YAEGASHI, H., and N. NISHIHARA. 1976. Production of the perfect stage in Pyricularia from cereals and grasses. Ann. Phytopathol. Soc. Japn. 42:511-515.