Mycoscience 36: 125–126, 1995

Note

Differential susceptibility of *Phialophora gregata* ff.sp. *adzukicola* and *sojae* to antimicrobial chemicals

Hideki Yamamoto

Akita Research Institute for Genetic Resources, Ohgata, Akita 010-04, Japan

Accepted for publication 7 December 1994

The susceptibility of *Phialophora gregata* ff.sp. *adzukicola* and *sojae* to antimicrobial chemicals was investigated. The minimum inhibitory concentrations (MICs) of benomyl, chloramphenicol, $CuSO_4$, cycloheximide and perchlorate for mycelial growth were the same for the two formae speciales. The MIC of hygromycin against f.sp. *adzukicola* was slightly lower than that against f.sp. *sojae*, and the latter was more resistant to iprodion than the former. Susceptibility to nystatin was markedly different: ff.sp. *adzukicola* and *sojae* had relative growth values of 3–20% and 59–93% at 100 μg/ml, respectively, and this difference could be used to differentiate the two formae speciales.

Key Words——antimicrobial chemical; *Phialophora gregata* f.sp. *adzukicola*; *Phialophora gregata* f.sp. *sojae*; susceptibility.

The soil-borne fungus *Phialophora gregata* (Allington et Chamberlain) Gams has been divided into three groups based on the restriction fragment length polymorphisms (RFLPs) of mitochondrial (mt) DNA: f.sp. *adzukicola* from Hokkaido, f.sp. *sojae* from Hokkaido and f.sp. *sojae* from Akita Prefecture (Yamamoto et al., 1993). Since it has been reported that groups based on mtDNA RFLPs correspond to vegetative compatibility (VC) groups in other fungi (Jacobson and Gordon, 1990; Kuninaga and Yokosawa, 1992), VC among these three groups of *P. gregata* has become a matter of interest. Recently, nitrate-nonutilizing mutants have been used for studies on VC in fungi (Takehara, 1992). However, neither such mutants nor auxotrophs are suitable for *P. gregata*, since the fungus shows little growth on synthetic media. Rather, dominant markers such as drug resistance are required. A study was therefore begun to investigate susceptibility of the fungus to several antimicrobial chemicals for the purpose of generating drug-resistant mutants for use in studies on VC in *P. gregata*. During the study, it was noticed that *P. gregata* ff.sp. *adzukicola* and *sojae* differed in susceptibility to certain antimicrobial chemicals, and this is reported here.

Six isolates from different sources each of the two formae speciales were used. They were transferred from potato dextrose agar slants to soybean stem agar (SSA) plates, on which they grow regularly and produce concentric colonies. The medium was prepared by diluting an extract of 5 g of dried and macerated soybean stems to 1 L with distilled water, to which 20 g of agar was added. After three weeks of incubation at 24°C, mycelial disks were excised from colony margins and used as inocula. Mycelial growth at various concentrations of eight antimicrobial chemicals was measured after two weeks of incubation on SSA at 24°C. For testing $CuSO_4$, SSA buffered with citrate buffer (pH 4.0) (Yamamoto et al., 1990) was used for basal medium, since $CuSO_4$ decreases pH of SSA. Medium without added chemicals was used as control.

Minimum inhibitory concentrations (MICs) of six antimicrobial chemicals against isolates A59To of f.sp. *adzukicola* and S60KS of f.sp. *sojae* are shown in Table 1, where MIC is the lowest concentration that inhibits mycelial growth completely. There was no difference in the MICs of benomyl (Benlate wettable powder), chloramphenicol, $CuSO_4$, cycloheximide and perchlorate between the two isolates. Incidentally, the high resistance of the two isolates to $CuSO_4$ is noteworthy. All six isolates each of the two formae speciales grew as well in the presence of 1,000 μg $CuSO_4$/ml as in its absence (data not shown), which indicates that media amended with $CuSO_4$ may be effective for selective isolation of f.sp. *adzukicola*, as in the case of f.sp. *sojae* (Mengistu et al., 1991).

The MIC of hygromycin against isolate A59To was slightly lower than that against isolate S60KS (Table 1). For the six isolates each of ff.sp. *adzukicola* and *sojae* (data not shown), the MICs of hygromycin were $20 < \leqq 40$ and $40 < \leqq 60$ μg/ml, respectively. A marked difference between the two formae speciales was observed in the susceptibility to nystatin. As shown in Fig. 1A, the mycelial growth relative to that on control medium (relative growth value) of isolate A59To decreased rapidly with increase in nystatin concentration. The growth

Table 1. Minimum inhibitory concentration[a] (MIC) of antimicrobial chemicals against *Phialophora gregata* ff.sp. *adzukicola* (isolate A59To) and *sojae* (isolate S60KS).

Antimicrobial chemical	MIC (μg/ml)	
	A59To	S60KS
Benomyl	0.3<≦0.4	0.3<≦0.4
Chloramphenicol	4,000<≦6,000	4,000<≦6,000
$CuSO_4$	1,000≦	1,000≦
Cycloheximide	5<≦10	5<≦10
Hygromycin	30<≦40	40<≦50
Perchlorate	60,000≦	60,000≦

[a] The lowest concentration that inhibits mycelial growth completely.

was halved at 20 μg/ml, but the isolate showed slight growth at 500 μg/ml. Isolate S62Ha-3 of f.sp. *sojae* was more resistant, showing relative growth value of 45% at 500 μg/ml of nystatin. When growth of the six isolates each of the two formae speciales was tested in the presence of 100 μg/ml of nystatin, f.sp. *sojae* isolates showed relative growth values of 59-93% (6.5-11.5 mm/two weeks), whereas f.sp. *adzukicola* isolates showed values of only 3-20% (0.3-2.8 mm/two weeks). Similarly, isolate S62Ha-3 was more resistant to iprodion (Rovral wettable powder) than isolate A59To, which could grow at 500 μg/ml (Fig. 1B). At 300 μg/ml of iprodion, the six isolates each of ff.sp. *adzukicola* and *sojae* showed relative growth values of 5-21% (0.8-2.0 mm/two weeks) and 29-43% (3.0-5.8 mm/two weeks), respectively.

Differences were thus found in the susceptibility of the two formae speciales to certain antimicrobial chemicals, which suggests that they differ physiologically. In particular, the difference in susceptibility to nystatin could be used to differentiate the two formae speciales, since the procedure is easy and judgment can be made within two weeks of incubation. For selective isolation of each forma specialis of *P. gregata* from soil and their common host (mung bean), however, other antimicrobial chemicals are required which allow one forma specialis to grow to some extent at a concentration perfectly inhibiting the growth of the other.

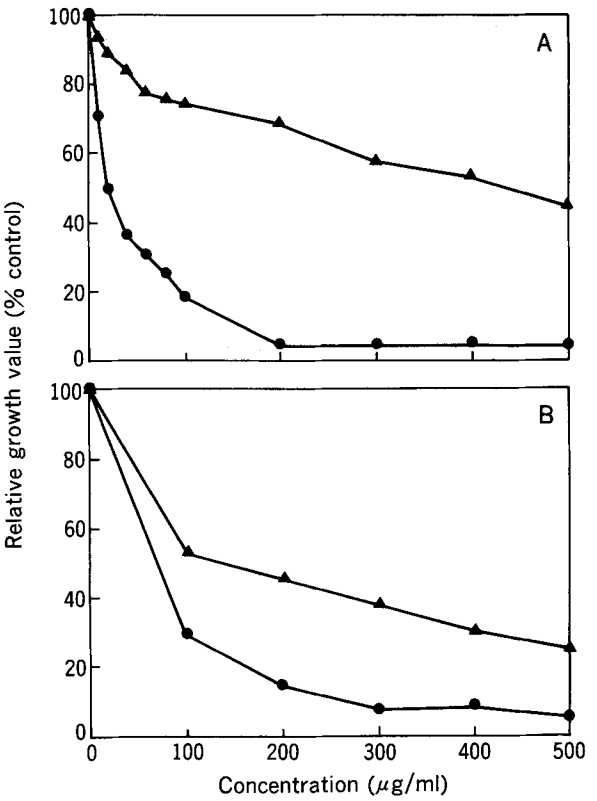

Fig. 1. Growth reduction of *Phialophora gregata* ff.sp. *adzukicola* (isolate A59To: ●) and *sojae* (isolate S62Ha-3: ▲) in response to nystatin (A) and iprodion (B). Relative growth value is mycelial growth at a given concentration of antimicrobial chemical relative to that on control medium.

Literature cited

Jacobson, D. J. and Gordon, T. R. 1990. Variability of mitochondrial DNA as an indicator of relationships between populations of *Fusarium oxysporum* f.sp. *melonis*. Mycol. Res. **94**: 734-744.

Kuninaga, S. and Yokosawa, R. 1992. Genetic diversity of *Fusarium oxysporum* f.sp. *lycopersici* in restriction fragment length polymorphisms of mitochondrial DNA. Trans. Mycol. Soc. Japan **33**: 449-459.

Mengistu, A., Tachibana, H. and Grau, C. R. 1991. Selective medium for isolation and enumeration of *Phialophora gregata* from soybean straw and soil. Plant Dis. **75**: 196-199.

Takehara, T. 1992. Nitrate-nonutilizing mutants of fungi and their use. Shokubutsu Boeki **46**: 395-399 (in Japanese).

Yamamoto H., Kobayashi, K. and Ogoshi, A. 1991. Comparison of culture characteristics of *Phialophora gregata* ff.sp. *adzukicola* and *sojae* in Japan. Trans. Mycol. Soc. Japan **32**: 529-533.

Yamamoto H., Kobayashi, K. and Ogoshi, A. 1993. Restriction fragment length polymorphisms in mitochondrial DNA of *Phialophora gregata* in Japan. Trans. Mycol. Soc. Japan **34**: 465-471.