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3 - 51 Thermotolerant Mutants of Entomopathogenic Fungi Obtained by Ion Beam- and Gamma Ray-induced Mutagenesis

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Entomopathogenic fungi, such as *Metarhizium anisopliae*, are economically important agents for integrated pest management (IPM) programmes of insect pests. However, negative effects of temperature, such as heat stress that exceeds 35 °C, can have serious deleterious effects on conidial germination and persistence, vegetative growth, sporulation, and/or the infection process in these entomopathogenic fungi. This may result in reduced effectiveness of these fungi in biological control, especially in hot seasons, tropical and subtropical regions, or in glasshouses with elevated temperatures. In this study, we attempted to enhance thermotolerance in a model entomopathogenic fungus, *M. anisopliae*, by mutagenesis using ion beams or gamma rays and evaluated the relative virulence of the resulting mutants compared with the wild-type isolates.

Two isolates of *M. anisopliae* (AcMa5 and PaMa02) were used as the wild-type isolates. Conidia of each wild-type isolate were irradiated with either carbon-ion beams ($^{12}C^{5+}$, 121.8 keV μm^{-1}) accelerated by an AVF cyclotron at TIARA, or with gamma rays (^{60}Co, 0.2 keV μm^{-1}) at Food Irradiation Facility, JAEA. Irradiated conidia were cultured at 38±1 °C (a temperature high enough to prevent the growth of both wild-type isolates) for 2 weeks to select for thermotolerant mutants.

Four mutants were isolated from the wild-type isolate, AcMa5; one mutant (AcMa5-ib) was from ion-beam irradiation (300 Gy) and three mutants (AcMa5-gr-1, AcMa5-gr-2, and AcMa5-gr-3) were from gamma-ray irradiation (100 or 1,000 Gy). From another wild-type isolate, PaMa02, one mutant (PaMa02-ib) was isolated as a result of ion-beam irradiation (100 Gy) and no mutants were obtained from gamma-ray irradiation.

For vegetative growth, all the mutants derived from wild-type AcMa5 had an upper thermal limit of 38 °C which was higher than that (36 °C) of the wild-type by 2 °C (Table 1). By contrast, mutant PaMa02-ib derived from wild-type PaMa02 had an upper thermal limit of 39 °C which was higher than that (36 °C) of the wild-type by 3 °C (Table 1).

Thermotolerance in conidial germination was evaluated after exposure of conidia to 45±1 °C for 0, 0.5, 1, and 3 h (Table 2). Conidia of the wild-type AcMa5 were unable to germinate after 1 h exposure, whereas all the mutants derived from it germinated (5.6-19.3%). By contrast, the mutant PaMa02-ib derived from wild-type isolate PaMa02 was, like the wild-type, unable to germinate after 1 h exposure, and also, its germination rate was not significantly different to the wild-type after 0.5 h exposure.

At 25 and 30 °C, most mutants were as virulent to the maize weevil adults as the wild-type, however, one mutant (PaMa02-ib) almost lost virulence entirely (data not shown). All mutants had no mutations in the neutral trehalase gene, β-tubulin gene nor ABC transporter gene (ifT1) which were previously associated with thermotolerance and fungicide tolerance in entomopathogenic fungi (data not shown).

In conclusion, ion beams and gamma rays are useful tools for improving biological characteristics, such as thermotolerance and fungicide-tolerance[1], in entomopathogenic fungi, but the mutants obtained in this study must be carefully evaluated for unpredictable negative side effects.

Reference

1) S. Shinohara et al., FEMS Microbiol. Lett. 349 (2013) 54.

Table 1 Colony diameter (mm, mean) of wild-type and mutant isolates at different temperatures.

Isolate	Temperature (°C)				
	30	33	36	38	39
AcMa5 (wild-type)	22.0	16.1	2.1	0	0
AcMa5-ib	27.9	18.9	4.9	0.7	0
AcMa5-gr-1	22.3	18.7	6.8	1.7	0
AcMa5-gr-2	25.2	16.0	6.1	2.3	0
AcMa5-gr-3	25.5	19.1	5.6	2.3	0
PaMa02 (wild-type)	26.0	15.7	0	0	0
PaMa02-ib	23.0	15.3	2.6	1.0	0

Table 2 Percent germination of wild-type and mutant isolates after exposure to heat stress of 45 °C.

Isolate	Hours of exposure to heat stress			
	0	0.5	1	3
AcMa5 (wild-type)	92.3	18.7	0	0
AcMa5-ib	93.0	66.1	19.3	0
AcMa5-gr-1	94.3	22.9	7.5	0
AcMa5-gr-2	96.0	45.9	5.6	0
AcMa5-gr-3	92.4	35.0	8.8	0
PaMa02 (wild-type)	91.6	16.0	0	0
PaMa02-ib	91.0	12.2	0	0