MECHANISM OF BILOGICAL SOIL DISINFESTATIONS

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Biological soil disinfestations (BSDs) are environmentally safe and used because of rising concerns on environmental risks of chemical soil fumigants. BSDs were developed as an alternative to chemical soil fumigations in Japan and uses organic materials such as wheat bran, rice bran, molasses, and ethanol (Shinmura 2000; Kobara 2007). These methods consist of application of organic matter, irrigation, and covering soil surface with conventional agricultural plastic films. Many soilborne pathogens such as *Fusarium oxysporum* f. sp. *lycopersici*, *Ralstonia solanacearum*, and *Meloidogyne incognita* were killed by BSDs (Shinmura 2004; Momma et al. 2005; Uematsu et al. 2007; Momma 2010). Its effectiveness accompanies drop in redox potential and accumulations of acetate and butyrate in soil, in addition to the evolution of peculiar odor (Momma et al. 2005; Momma et al. 2006; Kobara et al. 2007).

Soil amendment with acetic acid induced reductive condition and suppressed *F. oxysporum* f. sp. *lycopersici*. This event did not occur in autoclaved soil even when acetic acid was added. Soil reduction is considered to be one of the significant factors involved in the suppression of pests such as fungi and weeds in BSD-treated soil as is the case with flooded paddy soil. Our previous studies revealed that under reductive conditions, Fe^{2+} and Mn^{2+} increased in soil water, and their effect was subsequently investigated against *F. oxysporum* f. sp. *lycopersici*.

Bud cells of *F. oxysporum* f. sp. *lycopersici* were added to aqueous solution, containing either $FeSO_4$, $Fe_2(SO_4)_3$, $MnSO_4$, or $MgSO_4$ and incubated at 30°C for 7 days. Then, cell suspension was spread on potato dextrose agar to determine the rate of survived propagules; the pathogen was not affected by 1% $MgSO_4$ but decreased to below the detection threshold in 0.001% $FeSO_4$, 0.01% $MnSO_4$ and 0.1% $Fe_2(SO_4)_3$. The anaerobic (reductive) process in soil involves the evolution of Fe^{2+} and Mn^{2+}, and they were detected at 10^5 ppb and 10^4 ppb, respectively in BSD-treated soil. Thus, we considered that $Fe^{2+}$and Mn^{2+} were the principal factors of fungicidal

activity. Fe^{3+} was adsorbed to soil particles and little released to soil water.

When acetic acid, Fe^{2+} and Mn^{2+} were diluted to 0.001%, 0.0001%, and 0.0001%, respectively, their fungicidal activity was nullified. However, when acetic acid (0.001%) was combined with Fe^{2+} (0.0001%) but not with Mn^{2+} (0.0001%) suppression of *F. oxysporum* f. sp. *lycopersici* was reproduced, implying organic acids and metal ions such as Fe^{2+} play important roles coordinately in the fungicidal effect of BSD.

Conclusions and Remarks

· Bud cells of *F. oxysporum* f. sp. *lycopersici* were significantly suppressed in Fe^{2+} and Mn^{2+} solutions.

· Acetic acid facilitated fungicidal activity of Fe^{2+}.

· Mode of action of Fe^{2+} and Mn^{2+} should be clarified.

- Reactive oxygen species might be released through Fentonôs reaction. If reactive oxygen species are involved, what kind of molecules are responsible as radical source in BSD-treated soil?
- Fe^{2+} and Mn^{2+} disturb cell functions without reactive oxygen species? Silver ion itself disturbs cell membrane, some enzymes, and replication of nucleic acid. Can Fe^{2+} and Mn^{2+} cause such adverse effect?

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Table 1. Effect of BSD using ethanol on survival of chlamydospores of *Fusarium oxysporum* f. sp. *lycopersici*

	days				
	3	6	9	12	15
Control	—	—	—	—	5.0 (0.1)
water	3.1 (0.1)[1]	2.6 (0.2)	3.1 (0.1)	4.0 (0.0)	3.7 (0.0)
0.5% EtOH	3.0 (0.1)	3.5 (0.0)	ND[2]	ND	ND
1.0% EtOH	1.4 (0.2)	ND	ND	ND	ND
2.0% EtOH	ND	ND	ND	ND	ND

1: Log CFU/g dry matter (±S.E.), 2: Not Detected

Table 2. Effect of metal ions on survival of *Fusarium oxysporum* f. sp. *lycopersici*

Ion [Ingredient]	Concentration (%, v/v)			
	1	0.1	0.01	0.001
Fe^{2+} [$FeSO_4$]	ND[1]	ND	ND	ND
Mn^{2+} [$MnSO_4$]	ND	ND	ND	1.9 (0.1)[2]
Fe^{3+} [$Fe_2(SO_4)_3$]	ND	ND	3.8 (0.0)	4.3 (0.0)
SO_4^{2-} [$MgSO_4$]	4.8 (0.0)	4.9 (0.0)	4.9 (0.0)	4.9 (0.0)

1: Not Detected, 2: Log CFU/ml (±S.E.)

Table 3. Effect of acetic acid amendment on suppressive effect of metal ions against *Fusarium oxysporum* f. sp. *lycopersici*

$FeSO_4$ (0.0001%)	$MnSO_4$ (0.0001%)	Acetic acid (0.001%)	Incubation period (day)	
			4	8
—	—	—	4.8 (0.2)※	5.0 (0.0)
+	—	—	4.7 (0.0)	4.6 (0.0)
—	+	—	4.9 (0.0)	5.1 (0.1)
—	—	+	5.0 (0.0)	4.9 (0.0)
+	—	+	3.2 (0.1)	0.8 (0.8)
—	+	+	4.7 (0.1)	4.7 (0.0)
+	+	+	3.8 (0.1)	3.2 (0.4)

※Log CFU/ml (±S.E.)