Basic analysis of volatile organic compounds from actinomycetes

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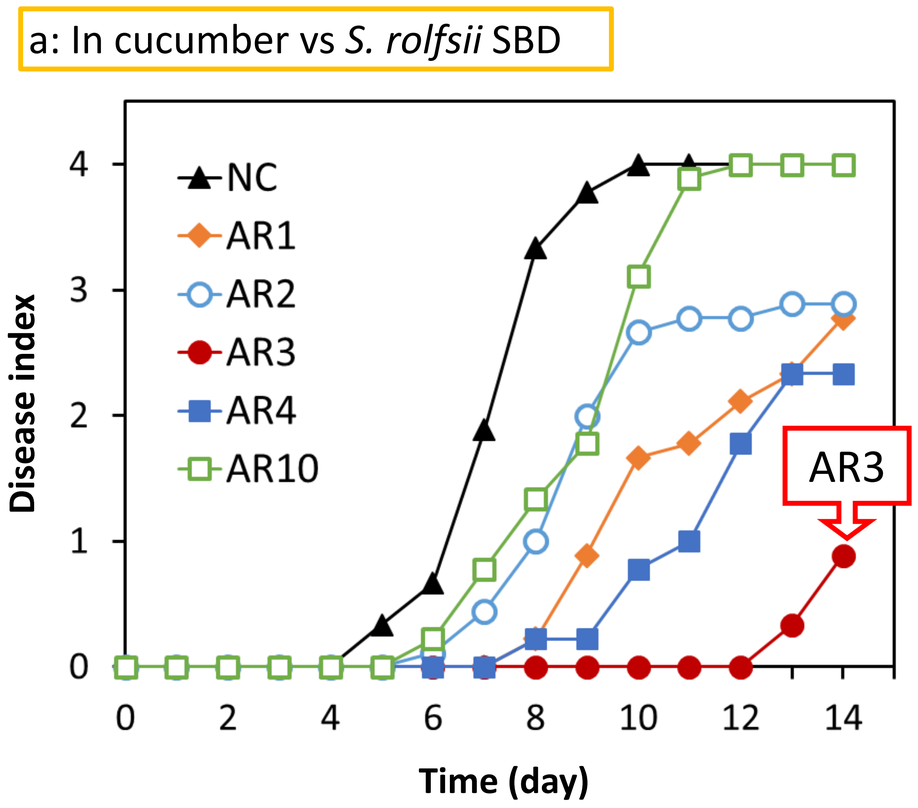
Introduction

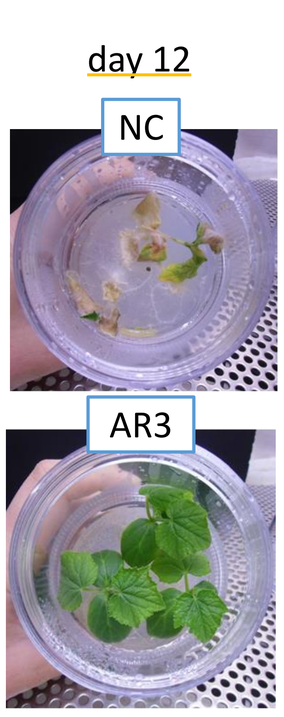
In recent years the food problem has become serious as the world population increases. As a consequence, further productivity improvement is required in agriculture. The control of phytopathogens which adversely affect crops, in particular, is indispensable for stable crop production. Currently, agricultural chemicals are mainstream as a means to do so, but also biocontrol agents having various effects are attracting attention. As a candidate of biocontrol agents, our laboratory has isolated actinomycetes with antimicrobial activity against multiple phytopathogens. These actinomycetes produced gaseous antimicrobial substances. In order to gain insights on volatile organic compounds (VOCs) produced from actinomycetes, we focused on actinomycetes AR3, AR4 and AR10 strains which were found to be effective in the infection control experiments.

Experiment 1. In vivo plant protection tests of actinomycetes

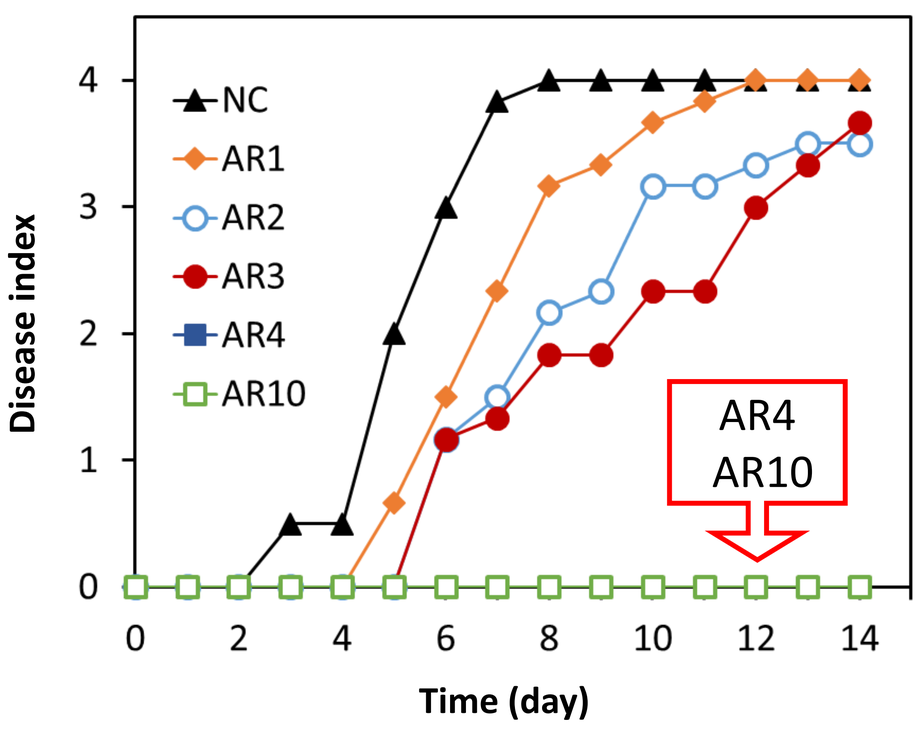
disease index		In cucumber vs. *Sclerotium rolfsii* SBD	In oasts vs. *Rhizoctonia solani* K1
0	light	no disease	no disease
1		browning of root	browning of root
2		browning stem	browning of the 1st leaf
3		loading of plants	browning of the 2nd leaf
4	heavy	plant death	plant death

a: In cucumber vs *S. rolfsii* SBD





b: In oats vs *R. solani* K1



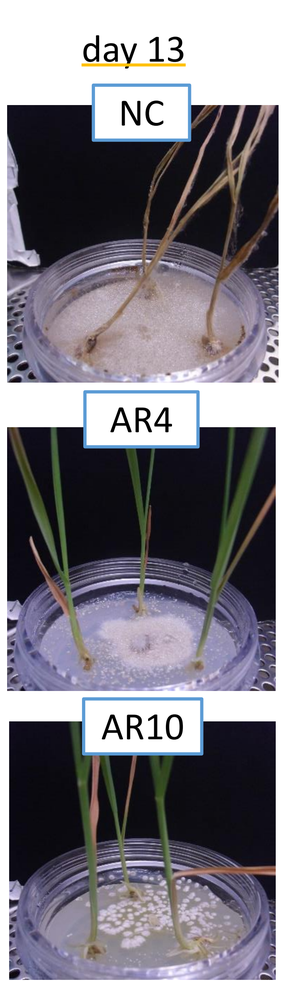
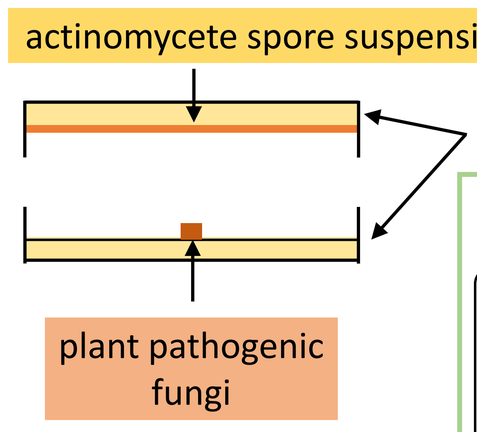


Fig. 1a, b Plant protection tests by actinomycetes against plant pathogenic fungi.

Experiment 2. Antifungal activity test using volatile organic compounds (VOCs) produced by actinomycetes



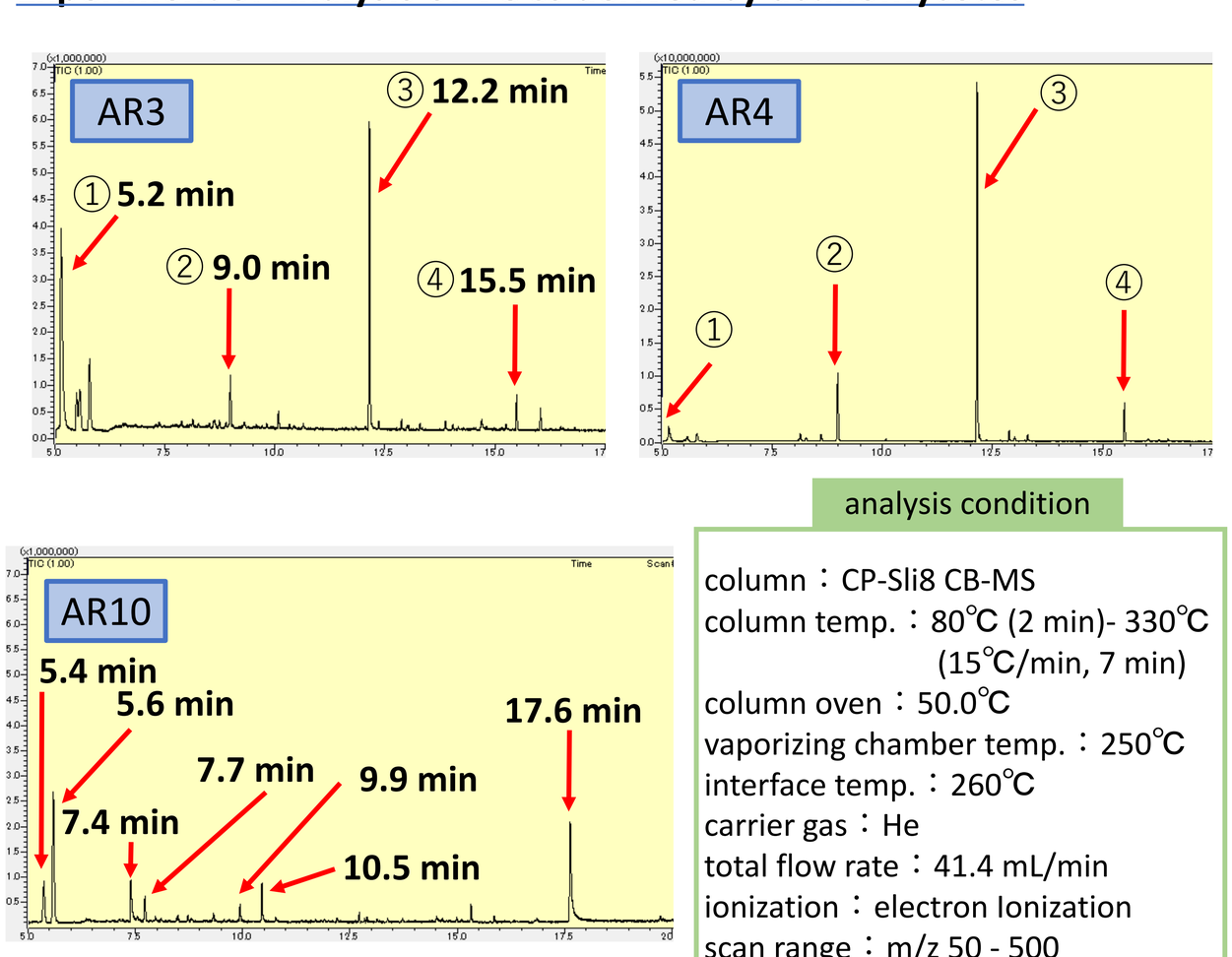
$$\text{inhibition rate}(\%) = \left(1 - \frac{\text{growth area of fungus with actinomycete } (cm^2)}{\text{growth area of fungus without actinomycete } (cm^2)}\right) \times 100$$

Table 1 Suppressive effect of VOCs from actinomycetes against plant pathogenic fungi.

plant pathogenic fungi	actinomycetes				
	AR1	AR2	AR3	AR4	AR10
Bipolaris oryzae	–	–	+++	+++	+++
Colletotrichum echinochloae	++	+++	+++	+++	++
Colletotrichum orbiculare	+	++	++	+	++
Fusarium oxysporum	+	++	+	++	+
Fusarium solani	++	++	+++	+	+
Monilinia fructigena	+++	+++	+++	+++	++
Phytophthora infestans	+	+	++	+++	+++
Rhizoctonia solani	+++	+++	+++	+++	+++
Rhizoctonia solani K1	+++	+++	+++	+++	+++

(-, inhibition late 0%; +, < 30%; ++, 30 - 69%; +++, ≥ 70%)

Experiment 3. Analysis of VOCs derived by actinomycetes



analysis condition

column：CP-Sli8 CB-MS
column temp.：80℃ (2 min)- 330℃ (15℃/min, 7 min)
column oven：50.0℃
vaporizing chamber temp.：250℃
interface temp.：260℃
carrier gas：He
total flow rate：41.4 mL/min
ionization：electron Ionization
scan range：m/z 50 - 500

Fig. 2 VOCs production by actinomycetes.

Conclusion

- The actinomycetes were isolated from the ant nest, and the strain AR3, AR4 and AR10 showed strong antifungal activity against plant pathogenic fungi *in vivo* plant protection test.
- The isolated actinomycetes produced VOCs, which showed an antifungal activity. Especially, strain AR3, AR4 and AR10 showed strongly antifungal activity than other isolates.
- As a result of GC-MS analyses, similar peaks were detected in strain AR3 and AR4. We are going to identify these peak of VOCs produced by strain AR3, AR4 and AR10.
- This study suggests that AR3, AR4 and AR10 would protect plants from plant pathogenic fungi.