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Selection of KYC 3270, a Cellulolytic Myxobacteria of *Sorangium cellulosum*, against Several Phytopathogens and a Potential Biocontrol Agent against Gray Mold in Stored Fruit

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During 2002–2008 in Korea, 455 extracts from myxobacteria consisting of 318 cellulolytic and 137 bacteriolytic myxobacteria were isolated, which were then screened for antifungal activity against the phytopathogens *Botrytis cinerea*, *Colletotrichum acutatum*, *Penicillium* sp., *Pyricularia grisea*, and *Phytophthora capsici*. 204 isolates had antifungal activity, causing both a clear zone due to blocked spore germination and inhibition of mycelial growth; most (199) were from cellulolytic (*Sorangium cellulosum*) and only five were from bacteriolytic myxobacteria. *B. cinerea*, the best controlled among the five tested pathogens, had a unique group of antifungal isolates of myxobacterial extracts compared to the other pathogens' groups. Among seventy-nine bioactive myxobacteria, four isolates, KYC 3130, KYC 3247, KYC 3248 and KYC 3270, were selected and all were cellulolytic. Liquid culture filtrates of these four myxobacteria were applied to tomato, cherry tomato, strawberry, and kiwi fruits 5 h before inoculation with gray mold conidia; then the treated fruits were placed in an airtight container and the experiment was repeated six to eight times. Incidence (%) of gray mold on fruit of the infected control treatment was 84–98%, whereas it was only 5–21% after the KYC 3270 treatment. After KYC 3270 treatment of the four fruits, mold control was 79–95%, which was highest among the filtrates and statistically the same as treatment with fludioxonil, a registered chemical against gray mold of stored fruits.

Keywords : antifungal activity, *Botrytis cinerea*, cellulolytic myxobacteria, strawberry

Myxobacteria have a fruiting body with bacteriolytic or cellulolytic characteristics (Cho, 2001; Dawid, 2000; Kim and Son, 2001; Kim et al., 2003a). The cellulolytic myxobacteria are able to degrade cellulose, grow well, and can be isolated in culture medium containing cellulose. So far, two

species of cellulolytic myxobacteria are known, *Sorangium cellulosum* and *Byssosvorax cruenta* (Reichenbach, 2001). *S. cellulosum* produces more than 30 different bioactive metabolites, including ambruticin, chivosalazole, disorazole, eliamid, epothilone, etnangien, jerangolid, ratjadon, socein, sorangicin, soraphen, spirodienal, spirangien, and tartrolon, which comprise 48% of the bioactive metabolites of myxobacteria (Dawid, 2000; Gerth et al., 2003; Niggemann et al., 2002).

Most bioassays for biological activity of myxobacteria have been with bacteriolytic myxobacteria such as *Myxococcus* spp. (Ahn and Kim, 2002; Bull et al., 2002; Chung et al., 2008; Kim and Yun, 2011; Kim et al., 1998; Kim et al., 2003b; Krug et al., 2008), although the antifungal activity of *S. cellulosum*, a cellulolytic myxobacterium, against several phytopathogenic fungi has been examined in Germany (Reichenbach and Höfle, 1994). Soraphen and epothilone are two natural products of *S. cellulosum*, and soraphen in particular is an antifungal compound. In field trials, this compound completely inhibited apple scab fungi (*Venturia inaequalis*) and also prevented grape gray mold (*Botrytis cinerea*) (Reichenbach and Höfle, 1994). According to the paper disk method for dual culture, Spirodienal C, new metabolites from *S. cellulosum*, have antifungal activity against *B. cinerea*, *Botryosphaeria dothidea*, and *Sclerotinia sclerotiorum* (Ahn, 2009). Hence, such a cellulolytic myxobacterium has extraordinary ability to produce secondary metabolites with biological activity (Gerth, 2003); however, more cellulolytic myxobacteria containing similar natural products have yet to be studied.

B. cinerea is a phytopathogen on more than 200 species under humid and low temperature conditions during storage (Hang et al., 2005; Kulakiotu et al., 2004; Tian et al., 2002; Williamson et al., 2007). To control *B. cinerea* in Korean fields, many fungicides having different modes of action were developed, e.g., benzimidazole, dicarboximide, sulfamide, *N*-phenylcarbamate, phenylpyrrol, and anilinopyrimidine (Choi et al., 1999, 2009a). However, A *B. cinerea* resistance to benzimidazole through a change of amino acid residue in β -tubulin was discovered (Soung and Sung,

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2007). A new procymidone fungicide was developed but this led to several new *B. cinerea* strains with single and multiple resistances (Lee et al., 2007). Because *B. cinerea* is able to change during hyphal fusion, genetic changes are frequent, often leading to resistance to fungicides (Choi et al., 1995). If an edible product such as strawberry contains fungicide residues, this leads to public concern, which has thus accelerated the search for alternative control strategies against gray mold, e.g., biological controls (Hjeljord et al., 2001).

For biological control of *B. cinerea*, *Acremonium strionum* (Choi et al., 2009b), *Bacillus* spp. (Hang et al., 2005; Kim et al., 2007; Park et al., 2006; Son et al., 2002), *Pseudomonas* spp. (Sharma et al., 2009), *Trichoderma harzianum* (Hjeljord et al., 2002; O'Neill et al., 1996), *Ulocladium atrum* (Boff et al., 2002; Lee et al., 2004), and natural volatiles (Kulakiotu et al., 2004) have been applied. The biopesticide for *B. cinerea* control, Trichodex[®], is a commercial product from *T. harzianum* containing an enzyme that dissolves the fungal cell wall and competes for nutrients with the pathogen. One can also delay *B. cinerea* conidial germination and prevent infection into host tissues (Paulitz and Bélanger, 2001).

Since myxobacteria are producing unique secondary metabolites as earlier mention, their biological activity could provide alternative biocontrol agents against gray mold in various storage fruits, thus reducing the need for chemical treatments. Biocontrol activity against gray mold by *S. cellulorum*, a cellulolytic myxobacterium, has never been investigated on stored fruits such as strawberry, tomato, and kiwi. We screened 455 myxobacteria from Korean soil for antifungal activity against several phytopathogens including *B. cinerea*, and compared antifungal activity against gray mold among the selected cellulolytic myxobacteria, KYC 3130, KYC 3247, KYC 3248 and KYC 3270, on tomato, cherry tomato, strawberry and kiwi.

Materials and Methods

Myxobacterial extracts. In total, 455 myxobacterial extracts were obtained from Myxobank, which had been isolated from South Korean soils between 2002 and 2008. Among these, 318 were cellulolytic and the rest were bacteriolytic myxobacteria. All 318 cellulolytic myxobacteria were identified as *S. cellulorum*, while the 137 isolates of bacteriolytic myxobacteria were identified as *Myxococcus* spp., *Corallococcus* spp., *Stigmatella* spp., and *Citrobacter* sp. (Fig. 1). *S. cellulorum* extracts were prepared with ST21CXP medium (Hyun et al., 2009), and extracts of bacteriolytic myxobacteria were prepared with WCX medium and *E. coli* as feedstock (Park et al., 2004). Either ST21CXP or WCX medium was used for the

Fig. 1. The profile of 455 myxobacterial extracts screened for antifungal activity from Myxobank. The only species of cellulolytic myxobacterium among 318 extracts was *Sorangium cellulorum*. The species of bacteriolytic myxobacteria in the other 137 extracts included *Myxococcus* spp., *Corallococcus* spp., *Stigmatella* spp., and *Citrobacter* sp., among others.

development of fruiting bodies, and crystals were obtained from 50 ml of liquid medium. The crystals were dissolved in dimethyl sulfoxide (DMSO) to obtain 1 ml of myxobacterial extract.

Phytopathogens. The fungal pathogens *B. cinerea* (no. 43528), *Pyricularia grisea* (no. 40420), and an oomycete pathogen, *Phytophthora capsici* (no. 40476), were obtained from the Korean Agricultural Culture Collection (KACC). *Colletotrichum acutatum* SM017 was isolated from field-grown hot pepper at Asan, Chungnam; *Penicillium* sp. was isolated from a diseased orange.

Antagonism to mycelial growth and conidial germination *in vitro*. A dual culture assay for antagonism was conducted on potato dextrose agar (PDA, Difco, Sparks, MD) (24 g potato dextrose, 15 g agar per liter) against the phytopathogens *B. cinerea*, *P. capsici*, *C. acutatum*, *Penicillium* sp., and *P. grisea* with antagonistic myxobacterial extracts for 7, 7, 10, 12, and 12 days, respectively. Mycelial agar plugs of 5 mm diameter of the five pathogens were placed in the middle of the PDA medium; then 12 µl of myxobacterial extract was put on the mycelia for 4–8 days at 25 °C and mycelial growth measured. Each replication consisted of three plates. Except for *P. capsici*, the clear zone around conidia *B. cinerea*, *C. acutatum*, *Penicillium* sp., and *P. grisea* was examined to assess blocking of germination by myxobacterial extracts. A conidial suspension of *P. grisea* was cultured in oatmeal agar (Difco, Sparks, MD) and the other three fungi were cultured in PDA. The conidial suspensions were adjusted to 1×10^5

conidia/ml. To test for germination, a 25 μ l conidial suspension of each fungus was smeared on PDA plates, then an 8-mm paper disk with 12 μ l myxobacterial extract was placed in the middle of the culture and incubated in darkness for 4 days at 25 °C. The control was 12 μ l DMSO on an 8-mm paper disk for each fungal culture. The diameter of the clear zone (no conidial germination) was measured. Among the tested myxobacterial extracts, those with the best antifungal activity were selected based on least mycelial growth and largest clear zone.

Cellulolytic myxobacteria. Based on mycelial growth and spore germination, four cellulolytic myxobacteria, KYC 3270, KYC 3248, KYC 3247 and KYC 3130 were selected. KYC 3270 and 3248 were isolated from soil at Gimcheon, Gyeongbuk, in 2008; KYC 3247 was isolated from soil at Hadong, Gyeongnam, in 2008; and KYC 3130 was isolated from soil at Hwacheon, Gangwon, in 2007. All were obtained from Myxobank [http://www.myxobank.or.kr/]. To compare antifungal activity between cellulolytic and bacteriolytic myxobacteria, *Myxococcus* sp. KYC 1126 with antagonistic activity against pepper anthracnose (Kim and Yun, 2011) was also cultured and applied as a liquid filtrate. The four cellulolytic myxobacteria were cultured on ST21P agar medium (1 g K_2HPO_4 , 0.02 g yeast extract, 1 g KNO_3 , 1 g $MgSO_4 \cdot 7H_2O$, 0.2 g $FeCl_3$, 0.1 g $MnSO_4 \cdot 7H_2O$, 15 g agar per liter) with the mycobiont on filter paper (Avantec no. 1, Toyo Roshi Kaisha, Japan) for 7 days at 32 °C. In addition, 1 ml of microelements (100 mg $MnCl_2 \cdot 4H_2O$, 20 mg $CoCl_2$, 10 mg $CuSO_4$, 10 mg $Na_2MoO_4 \cdot 2H_2O$, 20 mg $ZnCl_2$, 5 mg $LiCl$, 5 mg $SnCl_2 \cdot 2H_2O$, 10 mg H_3BO_3 , 20 mg KBr , 20 mg KI , 8 mg EDTA, $Na-Fe^{3+}$ salt (trihydrate) per liter; Hyun et al., 2009) was added to 1 l ST21P agar medium.

To produce antifungal material from the myxobacteria in liquid culture, the cell-cultured filter paper was inoculated in CSG/1 liquid medium (0.5 g glucose, 3 g soluble starch, 1 g raffinose, 2 g casitone, 0.5 g $MgSO_4 \cdot 7H_2O$, 1 g $CaCl_2 \cdot 2H_2O$, 0.25 g K_2HPO_4 , 0.5 mg vitamin B_{12} , 4 ml microelement solution per liter) for 7 days at 32 °C, with shaking at 180 rpm. The microelement solution for CSG/1 liquid medium was 10 ml H_2SO_4 , 14.6 g $FeCl_3 \cdot 6H_2O$, 2 g $ZnCl_2$, 1 g $MnCl_2 \cdot 4H_2O$, 0.43 g $CuCl_2 \cdot 2H_2O$, 0.31 g H_3BO_3 , 0.24 g $CaCl_2 \cdot 6H_2O$, 0.24 g $Na_2MoO_4 \cdot 2H_2O$ per liter (Hyun et al., 2009). For long-term maintenance, the mycobiont of the cellulolytic myxobacteria was prepared on filter paper on ST21P medium. The fruiting body of the cellulolytic myxobacteria was dried in a tube for 2 days in a desiccator, then put in a freezer at -70 °C. The preserved culture was revived when needed.

Bioassays of myxobacterial filtrates on fruit. A bioassay

for antifungal activity of the four cellulolytic myxobacteria (KYC 3270, KYC 3248, KYC 3247, and KYC 3130) and bacteriolytic myxobacterium (KYC 1126) was conducted on strawberry, tomato, cherry tomato, and kiwi. The surface of treated fruits was first sterilized with 1% sodium hypochlorite for 2 min, washed twice with sterilized water, then dried. Culture filtrates of the four cellulolytic and one bacteriolytic myxobacterium without dilution were sprayed on fruit until the filtrate ran off. To compare activity against gray mold on the stored fruit, the commercial fungicide fludioxonil was selected as a positive control and sprayed with 0.5 mg/ml. Fludioxonil (a.i. 20%, SC) is known to inhibit mycelial growth and spore germination. The treated fruits were dried for 5 h at 4 °C, and then 1×10^5 conidia/ml suspension was inoculated onto treated fruits, which were placed in a container with 100% RH by adding 20 ml distilled water on paper towel at 22 °C for 5–11 days, depending on the fruit.

Strawberry, tomato, and cherry tomato were inoculated with conidial suspensions of gray mold without any wounding, while kiwi was inoculated with a syringe needle at two wounds per fruit. Bioassay of strawberry, tomato, cherry tomato, and kiwi was replicated 6, 8, 8, and 7 times, respectively. Gray mold on strawberry, tomato, and cherry tomato was observed at 3, 4, and 5 days after inoculation; disease of kiwi was checked using fruit cross-sections 11 days after inoculation. The incidence of gray mold and control value for the myxobacterium as well as the fungicide were determined as follows:

$$\text{Gray mold incidence (\%)} = \frac{\text{No. of diseased fruits}}{\text{Total no. of treated fruits}} \times 100$$

$$\text{Control value (\%)} = \frac{(\text{Incidence on untreated plot} - \text{Incidence on treated plot})}{(\text{Incidence on untreated plot})} \times 100$$

Statistical multiple comparisons to the control values among the tested myxobacterial isolates and fludioxonil were analyzed with Fisher's least significant difference (LSD) test at a specified value of $\alpha < 0.05$.

Results

Myxobacterial extracts with antifungal activity. The myxobacterial extracts that completely inhibited mycelial growth and produced a clear zone by blocking germination were considered to have most antifungal activity (Fig. 2). Depending on the pathogen, the number of extracts with antifungal activity differed. Of 318 cellulolytic extracts, 74, 53, 43, and 29 extracts had antagonistic activity against *B. cinerea*, *P. grisea*, *Penicillium* sp., and *C. acutatum*, respectively (Fig. 2A). Of 137 bacteriolytic extracts, only five had antifungal activity specifically against *B. cinerea* (Fig. 2B).

Fig. 2. The number of extracts showing an antifungal activity among 318 cellulolytic (A) and 137 bacteriolytic (B) myxobacterial types against five phytopathogens. The antifungal activity on dual culture was to be seen as a clear zone (□) due to inhibition of conidial germination or complete lack of mycelial growth (■). Isolates (▒) that simultaneously showed a clear zone and mycelial growth inhibition. The tested five pathogens were *Botrytis cinerea*, *Pyricularia grisea*, *Penicillium* sp., *Colletotrichum acutatum*, and *Phytophthora capsici*. Inhibition of zoospore germination of *P. capsici* and a bioassay of bacteriolytic myxobacteria against *Penicillium* sp. were not conducted.

The cellulolytic myxobacteria had much more antifungal activity than the bacteriolytic myxobacteria, and *B. cinerea*

was the most affected pathogen in these tests. Among 77 extracts that completely inhibited *B. cinerea* mycelial growth, 74 blocked both *B. cinerea* germination and inhibited mycelial growth. Therefore, most extracts inhibited mycelial growth and blocked conidial germination in all pathogens examined (Fig. 2A). None of the 137 bacteriolytic myxobacteria extracts blocked *C. acutatum* conidial germination or completely inhibited its mycelial growth (Fig. 2B).

Antifungal effect on mycelial growth and spore germination of phytopathogens.

The effect of the antagonists on mycelial growth could be divided into three levels: complete, delayed, and non-inhibition (Table 1). Among the 318 cellulolytic myxobacterial extracts, about half had no antifungal activity, and delayed growth was little better than that of the complete level. However, the mycelial growth of *C. acutatum* was different: 44% was non-inhibited, 47% was delayed, and only 9% was completely inhibited. Among 137 bacteriolytic myxobacterial extracts, 115 extracts did not inhibit mycelial growth of *B. cinerea*, 7% completely inhibited mycelial growth, and 10% delayed growth of *B. cinerea*. Whereas 134 extracts didn't inhibit mycelial growth of *C. acutatum*, 0% completely inhibited the mycelial growth, and 2% delayed growth of *C. acutatum*.

Blocking of spore germination, as shown by a clear zone, was divided into five levels according to size (Table 1). Of 318 cellulolytic myxobacterial extracts, 53–85% of the 287 cellulolytic myxobacterial extracts tested against the four pathogens had no clear zone (Table 1). At best, 10% had clear zones of more than 30 mm against *B. cinerea*, which was the most inhibited fungus. Among the tested 137

Table 1. The number of myxobacterial extracts with antifungal activity against the five phytopathogens *Botrytis cinerea*, *Colletotrichum acutatum*, *Pyricularia grisea*, *Penicillium* sp., and *Phytophthora capsici*. Antifungal activity was assessed as the inhibition of mycelial growth and a clear zone due to blocking of spore germination of the pathogens

Myxobacterial type	Pathogen	Inhibition of mycelial growth			Clear zone due to blocking of spore germination				
		Complete inhibition	Delayed growth	Non-inhibition	>30 mm	20–30 mm	10–20 mm	<10 mm	None
Cellulolytic	<i>B. cinerea</i>	77/318 ^a	71/318	170/318	36/318	35/318	59/318	9/318	179/318
	<i>C. acutatum</i>	29/318	149/318	140/318	0/318	5/318	41/318	3/318	269/318
	<i>P. grisea</i>	53/318	87/318	178/318	40/318	22/318	25/318	0/318	231/318
	<i>Penicillium</i> sp. ^b	45/287	58/287	184/287	16/287	32/287	32/287	15/287	192/287
	<i>P. capsici</i>	43/318	65/318	210/318	NA ^c	NA	NA	NA	NA
Bacteriolytic	<i>B. cinerea</i>	10/137	12/137	115/137	0/137	0/137	6/137	2/137	129/137
	<i>C. acutatum</i>	0/137	3/137	134/137	0/137	0/137	0/137	0/137	137/137
	<i>P. grisea</i>	6/137	14/137	117/137	0/137	0/137	1/137	2/137	134/137
	<i>P. capsici</i>	6/137	12/137	119/137	NA	NA	NA	NA	NA

^a Number of myxobacterial isolates showing each character among the tested isolates.

^b Due to a shortage of extracts, some isolates of myxobacteria were not tested against *Penicillium* sp.

^c NA, not tested for blocking the germination of zoospores of *P. capsici*.

Table 2. Number of myxobacterial isolates with multiple antifungal activity on two phytopathogens simultaneously. Antifungal activity was the number of myxobacterial extracts that caused both inhibition of mycelial growth and a clear zone due to blocking conidial germination of the pathogen. The percentages in the parenthesis are proportion of extracts

Pathogens	Antifungal activity	No. of extracts with antifungal activity against two pathogens			
		<i>B. cinerea</i>	<i>P. grisea</i>	<i>Penicillium</i> sp.	<i>C. acutatum</i>
<i>B. cinerea</i>	79 ^a	–	17 (22%)	14 (18%)	7 (9%)
<i>P. grisea</i>	53 ^b	17 (32%)	–	27 (51%)	29 (55%)
<i>Penicillium</i> sp.	43 ^b	14 (26%)	27 (63%)	–	23 (54%)
<i>C. acutatum</i>	29 ^b	7 (24%)	29 (100%)	23 (79%)	–

^a Extracts with antifungal activity against *B. cinerea* consisted of 74 cellulolytic and five bacteriolytic myxobacteria.

^b The extracts with antifungal activity against each pathogen were all cellulolytic myxobacteria.

extracts of bacteriolytic myxobacteria, eight and three extracts blocked conidial germination of *B. cinerea* and *P. grisea*, respectively, with clear zones of 20 mm or less (Table 1). None of the 137 extracts inhibited *C. acutatum* conidial germination.

Multiple antifungal activity of myxobacterial extracts on the four phytopathogens. Some myxobacterial extracts had antifungal activity against more than one phytopathogen. Several extracts had antifungal activity simultaneously against two phytopathogenic fungi out of the four (*B. cinerea*, *P. grisea*, *Penicillium* sp., and *C. acutatum*; Table 2). Of the 79 extracts with antifungal activity against *B. cinerea*, 74 were cellulolytic and five were bacteriolytic myxobacteria. Among these, 17 shared antifungal activity against *P. grisea* and 14 shared it against *Penicillium* sp. All extracts with antifungal activity against *C. acutatum* also had antifungal activity against the combination *C. acutatum*-*P. grisea*. Combinations with *B. cinerea*, for example, with *P. grisea*, *Penicillium* sp., or *C. acutatum*, had antifungal activity of 9–32%. However, combinations without *B. cinerea*, for example, *P. grisea* with *Penicillium* sp. or *C. acutatum*, *Penicillium* sp. with *P. grisea* or *C. acutatum*, *C. acutatum* with *P. grisea* or *Penicillium* sp., had antifungal activity of 51–100% (Table 2).

Some myxobacterial extracts had antifungal activity against more than three pathogens simultaneously (Table

3). Twenty-three extracts had antifungal activity against *C. acutatum*, *P. grisea*, and *Penicillium* sp. simultaneously. KYC 3019, KYC 3188, KYC 3189 and KYC 3204 had antifungal activity against four pathogens tested. Clear zones against *C. acutatum* (14.5–21.4 mm) and *P. grisea* (32.7–37.1 mm) depended on the isolate, and those against *B. cinerea* were 12.1–18.3 mm. Despite 79 extracts having strong antifungal activity against *B. cinerea*, only four to seven extracts shared antifungal activity with other pathogens; hence, little antifungal activity against *B. cinerea* was shared among the other three pathogens.

Bioassay of selected myxobacteria against gray mold on stored fruit. After 3, 4 and 5 days of inoculation with gray mold conidial spraying, disease incidences on strawberry (Fig. 3A), tomato (Fig. 3B), and cherry tomato (Fig. 3C) were examined. The incidence of gray mold in the non-treated control was almost 100% 5 days after inoculation, while the incidence on kiwi (Fig. 3D) was about 80% 11 days after wound inoculation. Multiple comparisons among the tested isolates were conducted only on the final day of examination. The lowest disease incidence among the filtrate treatments was with KYC 3270 on all four fruits. This was statistically the same as the positive control with fludioxonil, and was significantly lower than any of the other tested myxobacteria isolates. Disease incidences after treatment with KYC 3248, KYC 3247, or KYC 3130, were

Table 3. Number of myxobacterial isolates with multiple antifungal activity against more than three phytopathogens simultaneously. The isolates with antifungal activity against the listed pathogen inhibited mycelial growth and produced a clear zone due to blocking spore germination of the pathogens in dual culture

Pathogens	No. of extracts with antifungal activity against more than 3 phytopathogens
<i>B. cinerea</i> + <i>C. acutatum</i> + <i>P. grisea</i>	7
<i>B. cinerea</i> + <i>C. acutatum</i> + <i>Penicillium</i> sp.	4
<i>B. cinerea</i> + <i>P. grisea</i> + <i>Penicillium</i> sp.	4
<i>C. acutatum</i> + <i>P. grisea</i> + <i>Penicillium</i> sp.	23
<i>B. cinerea</i> + <i>C. acutatum</i> + <i>P. grisea</i> + <i>Penicillium</i> sp.	4

Fig. 3. Incidence of gray mold on strawberry (A), tomato (B), cherry tomato (C), and kiwi (D) in airtight boxes treated with culture filtrates of myxobacteria. Except for kiwi, the incidence was measured 3 (□), 4 (▤), and 5 (▥) days after inoculation. Data bars are averages of eight replicates and error bars are standard errors. The same letters represent values that are not statistically different according to the LSD (Fisher's least significant difference) test at $P < 0.05$.

Table 4. Control values of the selected four cellulolytic and one bacteriolytic myxobacterium. Each value represents the average $\pm$ standard error of six to eight replicates, depending on the fruit. The letter following is the result of multiple comparisons by LSD test ($p < 0.05$) among the treatments

Fruits	Control value (%)					
	fludioxonil ^a	KYC 3270 ^b	KYC 3248 ^b	KYC 3247 ^b	KYC 3130 ^b	KYC 1126 ^c
Strawberry	88.5 $\pm$ 5.1d	84.7 $\pm$ 10.2d	49.7 $\pm$ 5.3bc	36.7 $\pm$ 3.0b	59.5 $\pm$ 6.7c	8.9 $\pm$ 5.9a
Tomato	72.5 $\pm$ 9.4c	79.2 $\pm$ 7.6c	35.4 $\pm$ 12.4b	29.2 $\pm$ 9.3b	46.2 $\pm$ 13.5b	22.9 $\pm$ 8.5ab
Cherry tomato	93.1 $\pm$ 2.1d	95.1 $\pm$ 2.5d	63.8 $\pm$ 5.2c	65.2 $\pm$ 6.9c	51.8 $\pm$ 7.0c	29.5 $\pm$ 6.3b
Kiwi	72.3 $\pm$ 5.2c	81.0 $\pm$ 4.6c	40.6 $\pm$ 10.2b	28.2 $\pm$ 9.4b	31.0 $\pm$ 10.2b	20.3 $\pm$ 8.5ab

^aA fungicide against *Batrytis cinerea*.

^bKYC 3270, KYC 3248, KYC 3247, and KYC 3130 are cellulolytic myxobacteria, i.e., *Sorangium cellulosum*.

^cKYC 1126 is a bacteriolytic myxobacterium, i.e., *Myxococcus* sp.

significantly lower than the infected control. Neither KYC1126, which was selected for anthracnose treatment (Kim and Yun, 2011), nor the bacteriolytic myxobacterium had antifungal activity on strawberry and tomato. Gray mold was found in negative controls (no artificial infection) on strawberry and tomato (41% and 15%, respectively). In contrast, disease incidences after KYC 3270 and fludioxonil treatment were 15.3% and 9.3%, respectively, and significantly less than the negative control. Therefore, KYC 3270 had a biological curative effect and fludioxonil had a chemical curative effect.

The control values of the four selected cellulolytic myxobacterial isolates were compared with a positive control, fludioxonil, and a bacteriolytic myxobacterial isolate (Table

4). Depending on the tested fruit, the control values of KYC 3270 isolates were 80–95%, which is statistically the same as with fludioxonil; values for controls of the other three cellulolytic myxobacteria were about 30–60%, which is statistically lower than in KYC 3270, while values for the bacteriolytic myxobacterium were 10–30%. Treatment with cellulolytic myxobacterial filtrates gave the best control in cherry tomato, followed by strawberry, while kiwi was the least controlled.

Discussion

Among the tested 455 myxobacterial extracts, 204 had antifungal activity that totally inhibited mycelial growth

and produced a clear zone by blocking spore germination on the tested phytopathogens. Only five bacteriolytic isolates out of these 204 had antifungal activity. Between 9% and 25% of 318 cellulolytic myxobacteria completely inhibited mycelial growth, depending on the pathogen, whereas only 0 to 7% of 137 bacteriolytic myxobacteria inhibited growth. In addition, the percentage of extracts that caused clear zones due to inhibited spore germination of three or four fungi was 15–44% for cellulolytic but only 0–6% for bacteriolytic myxobacteria. Although most research on bioactivity has been conducted on bacteriolytic myxobacteria (Bull et al., 2002; Chung et al., 2008; Kim et al., 1998; Kim et al., 2003b; Krug et al., 2008; Kim and Yun, 2011), more cellulolytic myxobacteria should be studied because they have superior antifungal activity to bacteriolytic myxobacteria.

Among the five tested phytopathogens, *B. cinerea* was the best controlled with 455 myxobacterial extracts. The clear zone due to blocking of *B. cinerea* spore germination was wider and inhibition of mycelial growth larger than with the other tested pathogens. Four isolates had antifungal activity against all tested phytopathogens, including *B. cinerea*, although over 70 cellulolytic myxobacteria produced a larger clear zone than these four isolates. Hence, the selection of myxobacterial isolates should best be against a targeted fungus (e.g., *B. cinerea*) rather than a spectrum-wide test against several pathogens. The antifungal group of isolates against *B. cinerea* differed from those for the other three fungi, while the groups of isolates against the other fungi were shared. Note that all 27 selected isolates with antifungal activity against *C. acutatum* also had antifungal activity against *P. grisea*.

In this experiment, KYC 3270 produced a 38-mm clear zone in *B. cinerea* mycelium; it controlled more than 95% gray mold on cherry tomato and 79–85% on strawberry, tomato, and kiwi. Park et al. (2006) reported that *Bacillus* spp. have anti-gray mold activity, with a 26-mm clear zone in dual culture; *Bacillus licheniformis* and *B. megaterium*, which were selected among 250 isolates, made 35-mm and 37-mm clear zones in *B. cinerea* and controlled 95% and 91% of gray mold, respectively, on green perilla (Son et al., 2002); a wettable powder formulation of *B. subtilis* from a strawberry leaf controlled 92.9% of gray mold on strawberry, and *Acremonium strictum* controlled 88% of gray mold on tomato (Choi et al., 2009b). The level of antifungal activity of *S. cellulosum* KYC 3270 against gray mold was similar to the previously tested *Bacillus* spp. and *A. strictum*.

Gerth et al. (2003) reported that Soraphen, a material developed from *S. cellulosum*, inhibited growth of *B. cinerea*, while Ahn (2009) showed that Spirodienal C, produced from *S. cellulosum*, inhibited mycelial growth of *B. cinerea*, *B. dothidea*, and *S. sclerotiorum* but did not

inhibit mycelial growth of *C. acutatum*, *P. capsici*, *R. solani*, *Fusarium oxysporum*, or *Pythium ultimum*. However, liquid filtrates of *S. cellulosum* KYC 3270 not only completely inhibited *B. cinerea*, but also *C. acutatum*, *P. capsici*, *P. grisea*, and *Penicillium* sp. We expect that the filtrate of KYC3270 contains at least 10 bioactive components, including Soraphen and Spirodienal C. In the future, KYC 3270 should be further analyzed. KYC 1126, which had no antifungal activity on *B. cinerea*, but was effective on *C. acutatum* (Kim and Yun, 2011), is also worthy of further study and should be compared with KYC 3270.

Since *B. cinerea* can latently infect fruits before harvest and produce conidia during storage or transport, infected fruits can become inocula for other fruits (Sharma et al., 2009). Selecting a biocontrol agent that has both preventive and curative effects after harvest is important. Two studies have reported that the curative effect of a biological agent is much less than the preventive effect (Choi et al., 2009b; Kim et al., 2007), although another reported that the preventive and curative effects of a biological agent are the same (Son et al., 2002). Because the filtrates were applied at least 12 h before inoculation with gray mold, antifungal activity of the tested isolates was focused on the preventive effect. A curative effect of KYC 3270 could be speculated from the strawberry bioassays, in which a 41.7% incidence in the negative control without *B. cinerea* inoculation suggests that the prepared strawberries were already infected. KYC 3270 inhibited *B. cinerea* mycelial growth and conidial germination, having both preventive and curative effects in the control of gray mold latent infection.

Even in developed countries, 20–25% of harvested fruit and vegetables are lost to postharvest pathogens (Sharma et al., 2009). Synthetic fungicides are used to control postharvest disease (El-Ghaouth, 2001), but safer and eco-friendly alternatives can provide better and more acceptable control. To enhance the bioefficacy of our selected myxobacterium requires consideration. First, the storage conditions (e.g., relative humidity, gas combination, and low temperature) should be examined. Second, our myxobacterium KYC 3270 should be further studied in mixtures; success in the control of gray mold with a mixture of *Aureobasidium pullulans* and *Bacillus subtilis* has been reported (Leibinger et al., 1997). Finally, salts, such as sodium bicarbonate, have been reported to enhance the control of gray mold on tomato (Xi and Tian, 2005). Thus, myxobacteria plus a salt may provide even better control of gray mold.

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