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ABSTRACT

Khan, N. I., Schisler, D. A., Boehm, M. J., Slininger, P. J., and Bothast, R. J. 2001. Selection and evaluation of microorganisms for biocontrol of Fusarium head blight of wheat incited by *Gibberella zeae.* Plant Dis. 85:1253-1258.

Gibberella zeae incites Fusarium head blight (FHB), a devastating disease that causes extensive yield and quality losses to wheat and barley. Of over 700 microbial strains obtained from wheat anthers, 54 were able to utilize tartaric acid as a carbon source when the compound was supplied as choline bitartrate in liquid culture. Four tartaric acid–utilizing and three nonutilizing strains reduced FHB in initial tests and were selected for further assays. Antagonists were effective against three different isolates of *G. zeae* when single wheat florets were inoculated with pathogen and antagonist inoculum. All seven antagonists increased 100-kernel weight when applied simultaneously with *G. zeae* isolate Z3639 ($P \leq 0.05$). *Bacillus* strains AS 43.3 and AS 43.4 and *Cryptococcus* strain OH 182.9 reduced disease severity by >77, 93, and 56%, respectively. Five antagonists increased 100-kernel weight of plants inoculated with *G. zeae* isolate DAOM 180378**.** All antagonists except one increased 100-kernel weight, and four of seven antagonists reduced disease severity ($P \leq 0.05$) when tested against *G. zeae* isolate Fg-9-96. In spray-inoculation experiments, *Bacillus* strains AS 43.3 and AS 43.4 and *Cryptococcus* strains OH 71.4 and OH 182.9 reduced disease severity, regardless of the sequence, timing, and concentration of inoculum application ($P \leq 0.05$), though 100-kernel weight did not always increase when antagonists were applied 4 h after inoculum of *G. zeae*. Overall, 4 of 54 isolates that utilized tartaric acid in vitro were effective against *G. zeae* versus only 3 of 170 isolates tested that did not utilize tartaric acid ($P \leq 0.05$, χ-square test of goodness of fit), demonstrating the potential benefit of prescreening candidate antagonists of FHB for their ability to utilize tartaric acid. Biological control shows promise as part of an integrated pest management program for managing FHB.

Additional keywords: *Fusarium graminearum*, scab of wheat

Fusarium head blight (FHB), also known as scab of wheat, pink mold, white-heads, and tombstone scab, is responsible for extensive damage of wheat in humid and semihumid regions of the world (2,12). The primary causal agent of scab of wheat in North America, *Gibberella zeae* (anamorph = *Fusarium graminearum*), can produce potent toxins, such as the estrogenic toxin zearalenone (8) and the trichothecene deoxynivalenol (DON, vomitoxin) (14,22). DON can inhibit amino acid incorporation and protein production in plant tissues (4), and grain heavily contaminated by the toxin is frequently unsuitable for human consumption and may be refused as feed (27). Infection of wheat kernels by *G. zeae* reduces grain yield and affects grain quality (5). The infection of seed reduces seed germination, seedling vigor, and plant emergence (3).

Chemical control and resistant cultivars are potential options for reducing the severity of FHB. Registered fungicides can be effective; however, residue and cost concerns are potential problems with chemical usage. All wheat cultivars currently in production are vulnerable to infection. Some success in controlling scab can be achieved by plowing fields after harvest to bury crop residues, but the preference for minimal-tillage agriculture renders this alternative less attractive (6). Biological control, though currently not available commercially for FHB, would offer another option for reducing the disease (11). The feasibility of biologically controlling this disease has been demonstrated (9,13,24).

Anthesis is a crucial time for the onset of FHB, with anthers promoting infection of wheat heads by *G. zeae* (25). Choline and betaine are found in wheat anthers and are stimulatory to the growth of conidial germ tubes of *G. zeae* (26). We surmised that microorganisms isolated from wheat anthers would be a good source of putative biocontrol agents and that information on carbon utilization by anther colonists could prove useful for selecting and formulating biocontrol strains. During our study, high-performance liquid chromatography (HPLC) analysis of culture broths containing individual anther colonists and choline in the form of choline bitartrate identified colonists capable of utilizing tartaric acid but was ineffective in identifying those capable of metabolizing choline. Fortuitously, a higher percentage of tartaric acid–utilizing microbes exhibited superior FHB biocontrol capabilities than did nonutilizing microbes. Tartaric acid is a compound that is poorly utilized by *G. zeae* as a carbon source (D. Schisler, *unpublished data*) and is a readily available byproduct in the processing of grapes and other fruits for juice and wine (1).

In previous investigations on biologically controlling plant disease, Schisler and coworkers (19) demonstrated that isolates of a fungal pathogen can differ substantially in their amenability to being suppressed with biocontrol agents. Whether biocontrol strains also vary in their relative effectiveness depending on the isolate of *G. zeae* used in a bioassay is not known. Additionally, the importance of the order and the timing of arrival of pathogen and biocontrol agent inoculum is not known for this biocontrol system.

Our objectives for this study were to (i) select putative antagonists of *G. zeae* based on obtaining microbes from anther tissue and assay colonists for their ability to utilize tartaric acid, (ii) screen putative antagonists for their efficacy against *G. zeae* and determine whether antagonist

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efficacy depends on pathogen isolate, and (iii) determine if the order or timing of pathogen and biocontrol inoculations impact the suppression of FHB.

MATERIALS AND METHODS

Isolation of anther colonists. In the spring of 1997, anthers were collected from flowering wheat plants across Illinois and Ohio, two states that had experienced recent FHB epidemics. Anthers were removed from wheat flowers using sterilized jeweler's forceps and placed in vials containing 10% (vol/vol) glycerol held at approximately 5°C. Vials then were frozen at –80°C. More than 400 anther collections were obtained. To isolate individual strains of microorganisms from anthers, vials were thawed until the glycerol suspension reached 4°C. Vials then were mixed using a vortex mixer for 30 s to liberate microorganisms from anther surfaces. Suspensions containing microorganisms were serially diluted using sterile, weak, pH 7.2 phosphate buffer (0.004% [wt/vol] KH_2PO_4 buffer with 0.019% [wt/vol] $MgCl_2$; Aid Pack, Gloucester, MA). Samples were plated onto a variety of solidified media (agar at 18 g/liter), including corn steep liquor (Solulys-AST at 10 g/liter [Roquette Corporation, Gurnee, IL], yeast extract at 1 g/liter, KH_2PO_4 at 2 g/liter, K_2HPO_4 at 2 g/liter, $MgSO_4 \cdot 7H_2O$ at 1 g/liter, NaCl at 0.1 g/liter, glucose at 15 g/liter, pH 6.8), malt yeast extract (yeast extract at 3.0 g/liter, malt extract at 3.0 g/liter, glucose at 10 g/liter, and peptone [type IV] at 5.0 g/liter, pH 3.5), and one-fifth strength Tryptic soy broth agar (TSBA/5, pH 6.8; Difco Laboratories, Detroit). The corn steep liquor medium was selected to represent a production medium that, in liquid state, could be economically feasible for commercial use. The TSBA/5 medium was selected as a general purpose medium that supports the growth of a wide variety of microorganisms, whereas the malt yeast extract medium was selected to isolate yeasts preferentially. Single colonies of antagonists showing distinct growth morphology were streaked for purity on TSBA/5. In all, 738 microbial isolates were purified and preserved in 10% (wt/vol) glycerol at –80°C until needed.

Stage 1 antagonist selection: tartaric acid utilization. All putative antagonists were tested for tartaric acid utilization as determined by HPLC analysis of antagonist culture broths containing choline bitartrate. Isolates were grown on TSBA/5 and harvested cells used to inoculate (optical density [OD] of approximately 0.8 at 620 nm wavelength light [A_{620}]) 10 ml of a minimal salt medium that contained choline bitartrate at 1 g/liter and urea at 1.26 g/liter. Cultures were incubated at 25°C and 250 rpm for 48 h in a shaker incubator. Colonized broths were centrifuged (5,000 rpm or approximately 2,000 relative centrifugal force (RCF) for 10 min at 4°C) and the supernatant was passed through 0.2-µm filters (Lida Manufacturing Corp, Kenosha, WI). For HPLC analysis of culture filtrates, a 30-by-4.6-mm cation H cartridge precolumn (Biorad, Hercules, CA), an Aminex HPX-87H, 300-by-7.8-mm column (Biorad), and a Waters 410 refractive index detector (Milford, MA) were used. The mobile phase for carrying filtrate sample was acidified H_2O (0.017N H_2SO_4) at a flow rate of 0.6 ml/min. Of the original 738 isolates of microorganisms assayed, 54 metabolized tartaric acid; all were selected for use in a two-head plant bioassay of biocontrol efficacy against FHB, as were an additional 188 randomly selected isolates that did not use tartaric acid.

Stage 2 antagonist selection: two-head plant bioassay. Two seedlings of hard red spring wheat (cv. Norm) per 19-cm-diameter pot were grown in an airsteam pasteurized (60°C for 30 min) potting mix (Terralite Redearth mix; W. R. Grace, Cambridge, MA) in a growth chamber (25°C, 14 h of light/day, 600 µmol/[m^2/sec]) for approximately 8 weeks prior to use in bioassays. Conidial inoculum of *G. zeae* isolate Z-3639 was produced on clarified V8 juice agar (CV8 agar) under fluorescent light at 12 h/day for 7 days at 24°C (17). Suspensions of macroconidia were obtained by flooding the surface of colonized CV8 agar with PO_4 buffer and dislodging conidia using a sterile inoculating loop. At 2 or 3 days before use, microbial strains were recovered from storage in 10% glycerol at –80°C by briefly warming vials at room temperature and streaking partially thawed glycerol onto TSBA/5. Cells were restreaked for purity after 48 h. Biomass of microbial strains was collected by rolling a sterile cotton swab on a 18- to 24-h culture on TSBA/5 and suspending the biomass in PO_4 buffer. Inoculum for wheat heads was prepared that contained antagonist suspension (OD approximately 0.2 to 0.3 at A_{620}, 5×10^5 conidia/ml and 0.04% [vol/vol] Tween 80; Sigma-Aldrich, St. Louis). To initiate the two-head plant bioassay for antagonists of *G. zeae*, the middle florets of two heads were coinoculated with 10 µl of a microbial suspension. Heads were inoculated within 2 to 4 days of flowering. Heads inoculated only with conidia of *G. zeae* served as controls. After inoculation, wheat plants were misted with water, incubated in a plastic humidity chamber for 72 h at approximately 22°C, and transferred to greenhouse benches. Plastic humidity chambers consisted of a PVC pipe frame covered with clear plastic. FHB severity was visually estimated using a 0-to-100% scale (23) 16 days after inoculation. Twenty-six strains, including nine tartaric acid-utilizers, prevented any visible FHB disease development and were selected for second-stage testing of efficacy against *G. zeae*.

Stage 3 antagonists selection: multiple-head plant bioassay. Management of wheat growth and preparation of *G. zeae* Z-3639 conidial inoculum were conducted as described above. Biomass of microbial strains was produced in liquid culture. Ten milliliters of semidefined complete liquid medium (20) in 50-ml Erlenmeyer flasks was inoculated to an OD of 0.10 at A_{620} with cells grown on TSBA/5 for 24 h. Flasks then were incubated in a shaker incubator at 250 rpm and 25°C for 48 h. At anthesis, 10 µl of an inoculum mixture of *G. zeae* (5×10^5 conidia/ml) and cells of a putative antagonist (48-h colonized broth diluted to 25% with PO_4 buffer; approximately 2×10^9 and 5×10^8 CFU/ml for bacteria and yeast strains, respectively) were used to inoculate single florets on 16 wheat heads (4 heads per replication, four replications/treatment). Plants were scored for disease severity after 16 days. Of the 26

Table 1. Antagonist strain designation and identification of bacteria and yeasts that reduce the severity of Fusarium head blight of wheat

Antagonist	NRRL accession no.[u]	Identification
AS 43.3	B-30210	*Bacillus subtilis/amyloliquefaciens*[v]
AS 43.4	B-30211	*Bacillus subtilis/amyloliquefaciens*[v]
OH 71.4	Y-30213	*Cryptococcus* sp. (syn. *Torula aurea*)[w]
OH 72.4	Y-30214	ND[x]
OH 131.1	B-30212	*Bacillus subtilis*[y]
OH 181.1	Y-30215	*Cryptococcus* sp. nov.[w]
OH 182.9	Y-30216	*Cryptococcus nodaensis* sp. nov.[z]

[u] NRRL patent culture collection, National Center for Agricultural Utilization Research, Peoria, IL.

[v] Identification by Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig, Germany, based on 16S rDNA sequence homologies and biochemical and physiological tests of taxonomic utility.

[w] Identification based on nucleotide sequence divergence in domain D1/D2 of large subunit 26S rDNA and on divergence in internal transcribed spacer (ITS) 1/5.8/ITS2 rDNA. (C. P. Kurtzman, *personal communication*).

[x] Yeast, not determined.

[y] Identification by MIDI Labs, Newark, DE, based on 16S rDNA sequence homologies and biochemical and physiological tests of taxonomic utility.

[z] Identification based on nucleotide sequence divergence in domain D1/D2 of large subunit 26S rDNA and on divergence in ITS 1/5.8/ITS2 rDNA. (C. P. Kurtzman, *personal communication*, and 16).

strains assayed, 7 reduced FHB severity by 25 to 80% ($P \leq 0.10$, *data not shown*) and were selected for further studies.

Antagonist bioassay against *G. zeae* isolates of different origin. Multiple-head plant bioassays were conducted as described above using the seven antagonists selected. Isolates of *G. zeae* used in the bioassays were Z3639, DAOM 180378, and Fg-9-96, originally isolated from Kansas, Ontario (Canada), and Ohio, respectively. Heads inoculated only with *G. zeae* conidia or only with PO_4 buffer served as controls. Disease severity was rated as described above and 100-kernel weight was determined after harvest. *G. zeae* was recovered via plating symptomatic tissue from randomly selected heads onto CV8 medium. There were four heads per replication and four replications per treatment distributed in a completely randomized design. Experiments with each isolate of *G. zeae* were performed at least twice. Data from repeated experiments were pooled because statistical analysis demonstrated that experiment interactions were rarely significant. Disease severity data were normalized when needed using the arcsine transformation before analysis of variance (ANOVA). Means were separated at $P \leq 0.05$ using Fisher's protected least significance difference test (PC SAS, ver. 6.12; SAS Institute, Inc., Cary, NC). Relative performance indices of efficacy ($RPI_{efficacy}$) were calculated for each antagonist performance against each isolate of *G. zeae*. For each experiment, an $RPI_{efficacy}$ value was calculated for each replication of each antagonist treatment. These values were used to calculate an average $RPI_{efficacy}$ for each antagonist strain and for ANOVA and mean separation of antagonist $RPI_{efficacy}$ averages. Relative performance indices allow different types of data to be compared using a standard scale and are dimensionless values that will theoretically range from 0 to 100, in which 100 = maximum antagonist efficacy relative to the other antagonist strains tested. Assuming FHB severity ratings are normally distributed, Z (the standard normal variate) will range from –2 to +2 (95% probability) when $Z = (X - \bar{x})/s$, where X is a single disease rating value observed for an antagonist strain, and $\bar{x}$ and s are the average and standard deviation, respectively, of all values obtained for all antagonist strains (21). $RPI_{efficacy}$ then is calculated as: $|(Z - 2)| \times 25$. For data sets where some Z values fall outside the range of –2 to +2, $RPI_{efficacy}$ is more accurately calculated as $(2 - Z) \times 25$.

Influence of order and timing of inoculum application on FHB disease. Management of wheat growth, microbial biomass preparation in liquid culture, and *G. zeae* conidial inoculum preparation were conducted as described above. A spray inoculation method was used to mimic the arrival of inoculum at the infection court in the field. An aqueous suspension (2 ml) containing *G. zeae* isolate Z3639 at 5×10^5 conidia/ml and 0.04% Tween 80 was applied as a mist (Spr• Tool; North American Professional Products, Woodstock, IL) over four wheat heads, immediately before or after and 4 h before or after treating heads with 5 ml of an aqueous suspension containing antagonist cells. Antagonist cells were applied at 10 or 50% of 48-h colonized broth. Heads sprayed only with conidia of *G. zeae* served as controls. Treatments were distributed in a completely randomized design ($n = 4$). Experiments were performed at least twice. Data analysis was as described above for separating treatment means from controls. Orthogonal contrasts provided analysis of the effect of antagonist concentration on FHB severity.

RESULTS

Antagonist selection. Approximately 7% (54 strains) of the strains recovered from anthers utilized tartaric acid in liquid culture. Tartaric acid–utilizers and an additional 188 randomly selected strains were used in replicated greenhouse plant bioassays to determine strain efficacy in reducing FHB. Twenty-six strains, including eight tartaric acid–utilizers, prevented the development of any visible FHB symptoms in two-head plant bioassays. Of the 26 strains tested (Table 1), 7, including 4 tartaric acid–utilizers, were superior in significantly reducing FHB disease in multiple-head plant bioassays ($P \leq 0.10$, *data not shown*). A higher proportion of tartaric acid–utilizing strains (4 of 54) were superior biocontrol strains than were nonutilizers (3 of 188) ($P \leq 0.05$, χ-square test of goodness of fit). None of the seven strains utilized choline in liquid culture (N. Khan, *unpublished data*).

Antagonist bioassay against *G. zeae* isolates of different origin. *Bacillus* strains AS 43.3 and AS 43.4, and *Cryptococcus* strain OH 182.9 (Table 1) reduced FHB disease severity by 77, 93, and 56%, respectively, in assays against *G. zeae* isolate Z3639 ($P \leq 0.05$, Table 2). All seven antagonists reduced FHB as indicated by increased 100-kernel weight of microbially treated wheat heads ($P \leq 0.05$, Table 2). Treatments with antagonist strains AS 43.3, AS 43.4, and OH 182.9 increased 100-kernel weight by 140, 144, and 100%, respectively. In bioassays against isolate DAOM 180378 of *G. zeae*, only strains AS 43.3 and AS 43.4 reduced disease as measured by either reduction in disease severity or disease incidence. Five strains increased 100-kernel weight, whereas *Cryptococcus* sp. OH 181.1 decreased 100-kernel weight (Table 2). Conversely, in bioassays using isolate Fg-9-96 of *G. zeae,* all antagonists except yeast OH 72.4 increased 100-kernel weight and four of seven antagonists reduced disease severity ($P \leq 0.05$, Table 2). Overall, strains AS 43.3 and AS 43.4 consistently reduced FHB disease regardless of the isolate of *G. zeae* used in the bioassays (Table 2). *Cryptococcus* spp. OH 71.4 and OH 182.9 reduced disease caused by all three isolates of *G. zeae* as quantified by at least one of the disease parameters measured. The remaining three antagonists were effective against two of the three isolates of *G. zeae* tested. Antagonist rankings based on $RPI_{efficacy}$ varied depending on the isolate of *G. zeae* (Table 3). Significant differences in the $RPI_{efficacy}$ values obtained for antagonist strains AS 43.4,

Table 2. Influence of microbial antagonists on Fusarium head blight incited by three isolates of *Gibberella zeae* on hard red spring wheat cultivar Norm[y]

	***G. zeae* isolate[z]**								
	Z 3639			**DAOM 180378**			**Fg-9-96**		
Treatment	**DS (%)**	**DI (%)**	**100-kw (g)**	**DS (%)**	**DI (%)**	**100-kw (g)**	**DS (%)**	**DI (%)**	**100-kw (g)**
G. zeae alone	90	95	1.5	76	91	1.8	54	66	3.2
Buffer alone	NT	NT	NT	0*	0*	4.2*	0*	0*	4.3*
AS 43.3	20*	63	3.6*	17*	41*	3.7*	3*	3*	4.0*
AS 43.4	6*	46*	3.9*	14*	31*	3.6*	11*	12*	3.8*
OH 71.4	78	82	1.9*	75	87	2.0*	3*	12*	4.0*
OH 72.4	82	89	1.8*	73	84	2.0*	51	56	2.8*
OH 131.1	79	89	2.1*	75	87	1.9	26*	34*	3.8*
OH 181.1	82	89	1.9*	88	91	1.7*	44	50	4.0*
OH 182.9	39*	72*	3.0*	69	84	2.0*	51	65	3.5*

[y] The middle floret of a central spikelet of a wheat head was coinoculated with 10 µl of a 48-h antagonist-colonized broth diluted to 25% with PO_4 buffer (10^8 to 10^9 CFU/ml) and *G. zeae* conidia (5×10^5 conidia/ml).

[z] DS = disease severity, DI = disease incidence, 100-kw = 100-kernel weight, NT = not tested. Within a column, means followed by an asterisk are significantly different from the *G. zeae* control (Fisher's protected least significant difference, $P \leq 0.05$).

OH 71.4, and OH 182.9 when assayed against the three isolates of *G. zeae* indicated that the relative efficacy of these antagonist strains was dependent on the pathogen isolate used to incite disease.

Influence of order and timing of inoculum application on FHB disease. In spray-inoculation experiments, all antagonists significantly reduced disease severity, regardless of the sequence, timing, and concentration of inoculum application ($P \leq 0.05$, Table 4), though some antagonists did not increase 100-kernel weight when inoculum of *G. zeae* was applied 4 h before the antagonist (Table 5). The comparison of antagonist concentration response as measured by orthogonal contrasts for disease severity indicated no significant effect of concentration for all the antagonists except AS 43.4 when *G. zeae* inoculum was applied 4 h before the antagonist (Table 6). When pathogen inoculum was applied immediately before or after antagonists, antagonist concentration influenced disease severity in some cases, though higher antagonist concentration was not consistently associated with greater reduction in disease severity (Table 6). All antagonists showed some significant differences in concentration effect on 100-kernel weight (Table 7), although increased antagonist concentration was not always associated with increased efficacy in reducing FHB disease. *Bacillus* strains were generally more effective in reducing FHB severity than were yeast isolates ($P \leq 0.05$, *data not shown*). *Bacillus* strains decreased FHB severity irrespective of their method of application on the wheat head (i.e., whether applied as a spray mist or via inoculation of single wheat florets). However, yeasts were more effective in suppressing FHB severity when applied as a mist than when applied to single florets (Tables 2, 4, and 5).

Table 3. Comparison of efficacy relative performance indices ($RPI_{efficacy}$) for seven antagonists when tested against three isolates of *Gibberella zeae* on flowering wheat heads[z]

	$RPI_{efficacy}$ for *G. zeae* isolates		
Treatment	**Z 3639**	**DAOM 180378**	**Fg-9-96**
AS 43.3	73.8 a	82.2 a	71.0 a
AS 43.4	84.4 a	84.6 a	64.2 b
OH 71.4	34.9 b	37.4 b	71.5 a
OH 72.4	32.3 a	38.9 a	28.9 a
OH 131.1	36.0 a	37.5 a	50.9 a
OH 181.1	31.4 a	27.3 a	34.8 a
OH 182.9	57.1 a	42.1 ab	28.7 b

[z] The hard red spring wheat cultivar Norm was used in greenhouse bioassays. $RPI_{efficacy}$ values were calculated using disease severity data from at least two experiments, each with four replicates per treatment. Within a row, means followed by the same lower-case letter are not significantly different (Fisher's protected least significant difference, $P \leq 0.05$).

Table 4. Percent Fusarium head blight disease severity when varying the time and sequence of pathogen and antagonist inoculum application to wheat heads

	Gibberella zeae (Z3639) inoculum applied[y]			
Antagonist[z]	**4 h before**	**Immed. before**	**Immed. after**	**4 h after**
None (control)	59	86	81	85
AS 43.3 (10%)	13*	2*	3*	21*
AS 43.3 (50%)	5*	1*	0*	15*
AS 43.4 (10%)	42*	3*	3*	30*
AS 43.4 (50%)	19*	33*	0*	18*
OH 71.4 (10%)	26*	24*	18*	49*
OH 71.4 (50%)	28*	51*	37*	63*
OH 182.9 (10%)	43*	64*	60*	45*
OH 182.9 (50%)	43*	49*	60*	58*

[y] Time before and after antagonist; Immed. = immediately. Within a column, means followed by an asterisk are significantly different from the control (Fisher's protected least significant difference, $P \leq 0.05$).

[z] Antagonists were applied as a mist at concentrations of 10 or 50% of a 48-h (early stationary growth phase) antagonist-colonized, semidefined complete liquid medium.

Table 5. One-hundred-kernel weights when varying the time and sequence of pathogen and antagonist inoculum application to wheat heads

	Gibberella zeae (Z3639) inoculum applied[y]			
Antagonist[z]	**4 h before**	**Immed. before**	**Immed. after**	**4 h after**
None (control)	1.8	1.6	1.7	1.4
AS 43.3 (10%)	2.4*	3.4*	3.4*	2.6*
AS 43.3 (50%)	2.6*	3.6*	3.1*	2.7*
AS 43.4 (10%)	2.5*	3.3*	3.2*	2.2*
AS 43.4 (50%)	1.7	3.0*	3.1*	2.6*
OH 71.4 (10%)	1.9	2.3*	2.8*	2.4*
OH 71.4 (50%)	2.4*	2.6*	2.5*	2.0*
OH 182.9 (10%)	1.9	2.3*	2.3*	2.7*
OH 182.9 (50%)	1.8	2.2*	2.3*	2.0*

[y] Time before and after antagonist; Immed. = immediately. Within a column, means followed by an asterisk are significantly different from the control (Fisher's protected least significant difference, $P \leq 0.05$).

[z] Antagonists were applied as a mist at concentrations of 10 or 50% of a 48-h (early stationary growth phase) antagonist-colonized, semidefined complete liquid medium.

DISCUSSION

Selection of putative antagonists for biological control of plant diseases usually involves collecting and screening large numbers of microbial isolates to enhance the probability of discovering a highly effective strain. Screening a large number of putative antagonists through the labor-intensive technique of plant bioassay may not be a feasible strategy for the initial selection of candidate antagonists if plant material is difficult to manipulate or produce. Therefore, various tests for the preselection of putative antagonists and the narrowing of the number of antagonists for bioassay on plants have been attempted, including in vitro antibiosis tests conducted on nutrient agar. However, the test medium used in these assays can influence the degree of antibiosis shown by putative antagonists (7,18). Additionally, the correlation between in vitro antibiosis on agar medium and biological control in situ is frequently inconsistent (10,15).

From a collection of 738 microorganisms obtained from wheat anthers, we selected 54 microbial strains that utilized tartaric acid and 188 that did not. As a relatively inexpensive byproduct from the production of grape and other fruit juices (1), tartaric acid has potential for use in formulations of tartaric acid–utilizing antagonists because the compound is poorly utilized by *G. zeae* (D. A. Schisler, *unpublished data*). Of 54 tartaric acid–utilizing isolates, 4 (7.4%) consistently reduced FHB disease severity and increased 100-kernel weights. In contrast, only 3 of 188 microbial isolates (1.6%) that did not utilize tartaric acid were successful in reducing FHB. This reduction in disease was obtained using microbial biomass produced in a liquid culture medium that should be affordable for use on a commercial scale. Testing candidate microbes for their ability to utilize tartaric acid may be a useful preliminary screen for narrowing the search for FHB antagonists. Employing this screening method substantially reduced the number of strains that advanced

to the labor-intensive step of plant testing, yet provided a high percentage of those strains that were effective. Though tartaric acid is present in many fruits, we are not aware of any reports of its presence in wheat tissues. We are currently investigating this possibility. Choline is present in wheat anthers and stimulates early germ tube growth of *G. zeae* (26). Surprisingly, none of the top FHB antagonists identified in this study utilized choline in liquid culture, though recently discovered strains with potential to biologically control FHB do (N. Khan, *unpublished data*).

The preliminary screening of putative antagonists also included recovering isolates from anthers on Solulys medium, a medium composed of relatively inexpensive nutrient sources that could be economically feasible for use in commercial production of microbial biomass. Additionally, biomass of putative antagonists was produced in liquid culture early in the selection process. Production of antagonists in liquid versus solid media and in liquid media of differing composition can affect the relative and overall performance of the antagonists produced (19). Even during the early stages of antagonist selection, therefore, antagonist biomass should be produced using a medium feasible for use in commercial production.

All seven of the antagonists chosen from the multiple-head plant bioassays reduced FHB disease symptoms incited by two of the three isolates of *G. zeae*, and four of the strains reduced symptoms incited by all three isolates of *G. zeae*. Interestingly, pathogen isolate influenced the relative efficacy of antagonist strains AS 43.4, OH 71.4, and OH 182.9 as determined by $RPI_{efficacy}$ values. It is not known whether this indicates that these strains will be more variable in performance than the other antagonists when tested in field environments where a diversity of *G. zeae* genotypes would be encountered. Differences in relative performance between potato dry rot antagonists when tested against a variety of isolates of *G. pulicaris* also have been reported (19).

Bacillus strains AS 43.3 and AS 43.4 consistently decreased FHB disease severity, increased 100-kernel weight, or both, regardless of the method of antagonist application utilized. Although determining the mode of action of antagonists was not part of this study, antibiosis may be a mechanism of biological control for strains AS 43.3 and AS 43.4 because they inhibited the growth of *G. zeae* in petri plate antagonism tests (N. Khan, *unpublished data*). The yeast isolates in this study did not demonstrate petri plate antagonism of *G. zeae* but were all utilizers of tartaric acid. Interestingly, all of the yeast isolates were more successful in reducing the severity of FHB when applied as a mist compared with when inoculated at a single point on a wheat head. This may be due to the fact that, in point inoculation, pathogen inoculum is placed within a floret, thereby directly providing an infection court to the pathogen and limiting the opportunity for yeasts to compete with *G. zeae* for nutrients before the pathogen has successfully invaded the host tissue. On the other hand, when sprayed on the external surfaces of the wheat head along with pathogen inoculum, yeasts may have an increased opportunity to compete for the nutrients present at the infection court, especially on anthers (25).

Although these antagonist strains were effective against three different isolates of *G. zeae* that were collected from three widely separated locations, it has yet to be seen how they will perform in field trials at different locations. Work will also be needed to enhance formulation technologies, including identifying additional compounds that could be used to preferentially enhance the activity of the biocontrol agents. In the absence of highly resistant cultivars or highly effective registered fungicides, this study confirms the potential of biological control as a viable option in an integrated pest management program for reducing scab of wheat.

Table 6. Orthogonal contrast analysis of the effect of concentration of antagonist on Fusarium head blight severity

	P **> F for *Gibberella zeae* (Z3639) inoculum applied[y]**			
Orthogonal contrast[z]	**4 h before**	**Immed. before**	**Immed. after**	**4 h after**
AS 43.3 (10%) vs. (50%)	0.197	0.893	0.652	0.496
AS 43.4 (10%) vs. (50%)	0.001	0.001	0.629	0.125
OH 71.4 (10%) vs. (50%)	0.848	0.001	0.007	0.115
OH 182.9 (10%) vs. (50%)	0.988	0.025	0.989	0.124

[y] Time before and after antagonist; Immed. = immediately.
[z] Antagonists were applied at concentrations of 10 or 50 % of a 48-h (early stationary growth phase) antagonist-colonized, semidefined complete liquid medium.

Table 7. Orthogonal contrast analysis of the effect of concentration of antagonist on 100-kernel weights of wheat heads inoculated with *Gibberella zeae* (Z3639)

	P **> F for *G. zeae* (Z3639) inoculum applied[y]**			
Orthogonal contrast[z]	**4 h before**	**Immed. before**	**Immed. after**	**4 h after**
AS 43.3 (10%) vs. (50%)	0.100	0.001	0.001	0.138
AS 43.4 (10%) vs. (50%)	0.001	0.001	0.786	0.001
OH 71.4 (10%) vs. (50%)	0.001	0.001	0.001	0.001
OH 182.9 (10%) vs. (50%)	0.144	0.031	0.997	0.001

[y] Time before and after antagonist; Immed. = immediately.
[z] Antagonists were applied at concentrations of 10 or 50 % of a 48-h antagonist-colonized, semidefined complete liquid medium.

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