Efficacy of Rhizobacteria as Biological Control Agents of Grassy Weeds

S.M. Boyetchko

Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, Saskatchewan

ABSTRACT

Deleterious rhizobacteria (DRB) associated with the roots and rhizosphere of weeds have tremendous potential as biological weed control agents. Screening and evaluation of the efficacy of DRB for biological control of downy brome (Bromus tectorum), green foxtail (Setaria *viridis),* and wild oats *(Avena fatua) was* conducted and methods for optimizing biocontrol activity were determined. Several hundred isolates of DRB were screened, with more than 100 isolates with 280% suppression of root growth in laboratory bioassays showing weed inhibitory properties. An increase in root growth suppression was demonstrated when unfiltered bacterial broth was incorporated into agar, compared to cell-free culture filtrate alone. Nutritional factors also played a role in enhancing biocontrol activity. Increases in root and shoot suppression were demonstrated when selected DRB isolates were grown in a nutrient broth, compared to a minimal salts broth. Specific requirements in the nutritional environment of the bacterial culture medium and the effect these changes have on propagule yield, efficacy and stability will have a significant impact on the potential of a biological control agent and its success as a commercial product.

INTRODUCTION

Throughout the world, weeds contribute significantly to reduced crop yield and quality even though management programs to control them with chemical herbicides have been developed (Swanton *et al.,* 1993; TeBeest et al., 1992). In Canada, average annual yield losses due to weeds were $984 million (Swanton et al., 1993). Although chemical herbicides are effective against many weeds, the dependence of the agri-food industry on chemicals has resulted in several problems, including herbicide-resistant weed populations, reduced soil and water quality, herbicide residues, and detrimental effects on non-target organisms (Beckie and Morrison, 1993; Heap et al., 1993). Over the last 20 to 30 years, plant pathologists and weed scientists have begun exploring the utilization of plant pathogens for biological weed control and their integration into weed management systems (Charudattan and DeLoach, 1988; TeBeest et al., 1992).

Inundative biological control of weeds with microbial agents offers an ecological alternative to chemical pest control products. The inundative strategy involves mass-production and application of a host-specific agent at high inoculum levels over a localized area infested with the target weed (Charudattan, 1991; TeBeest et al., 1992). Exotic as well as indigenous plant pathogens have been used; however, the majority of the biological agents investigated using this strategy have been indigenous pathogens. Weed control using this approach is relatively short-term and the biological control agent is not expected to be self-sustaining.

Historically, research has focused on foliar-applied pathogens for reducing weed populations, but, soil-borne microorganisms, such as rhizobacteria (root-colonizing bacteria), have recently been evaluated for control of grassy weeds. Deleterious rhizobacteria (DRB), associated with the roots and rhizosphere of weeds such as downy brome (Bromus *tectorum* L.), leafy spurge *(Euphorbia esula* L.), wild oats *(Avena fatua* L.) and green foxtail *(Setaria viridis* [L.] Beauv.), have caused significant inhibitory effects

to root growth and plant development (Boyetchko and Mortensen, 1993; Kennedy *et al.*, 199 1; Souissi and Kremer, 1994). Weed suppressive soils may contain a number of DRB, several of which produce secondary metabolites (e.g. phytotoxins). Therefore, these microorganisms may be used as potential biological control agents to control target weed species.

The objective of this study was to screen and evaluate the efficacy of rhizobacteria as biological control agents of downy brome, green foxtail, and wild oats and to determine methods of optimizing biological control activity under different nutritional conditions.

MATERIALS AND METHODS

Isolation and screening rhizobacteria for biological control

Roots of downy brome, green foxtail, and wild oats were collected from a number of soils in Saskatchewan. Rhizobacteria were isolated from roots of each weed species by placing root samples in 10 ml sterile distilled water in test tubes, shaking on a rotary shaker at 150 rpm for 30 minutes, serially diluting with sterile distilled water, and pipetting 1 ml from each dilution onto Sands and Rovira (SR) medium (Sands and Rovira, 1970), a selective media for fluorescent pseudomonads. All cultures were incubated in the dark at 15°C for 72 to 96 hours or longer, until colonies formed. Single bacterial colonies were randomly selected and restreaked onto the SR medium several times in order to obtain single, purified isolates.

For screening, purified rhizobacterial isolates were grown in a minimal salts broth culture, the supematant was collected and incorporated as cell-free culture filtrate (CFCF) or as unfiltered centrifuged supematant (UNF) into Difco Bacto agar (45°C) at concentrations of 10% or 30% (v/v) and poured into Petri plates. Controls consisted of broth culture (minus bacteria) incorporated into the agar. Seeds of each weed species were surface sterilized and biocontrol activity of each bacterial isolate was evaluated by placing the seeds at one end of the bioassay plate and incubating in the dark at 15°C. After 7 days, root growth was recorded.

Effect of nutritional conditions on biocontrol activity

Two rhizobacterial isolates (GF-10 and GF-2) demonstrating biocontrol activity against green foxtail were selected and grown in 3 types of bacterial culture media: Minimal Medium (PMM), Nutrient Broth (NB) and Trypticase Soy Broth (TSB); a water control was used for comparison. Agar bioassays with CFCF were conducted as described above and root suppression after 7 days was evaluated.

In addition, green foxtail seeds were bacterized (coated with bacteria) with each of the two test isolates grown in either PMM, NB, or TSB, and a water control, placed in growth pouches for 7 days, after which root and shoot growth were evaluated.

RESULTS AND DISCUSSION

Isolation and screening rhizobacteria for biological control

The rhizobacterial isolates tested exhibited a wide range of activities on all three grass weeds (Tables l-3); some are deleterious rhizobacteria (DRB), showing inhibitory effects to root growth while other isolates are plant growth promoting rhizobacteria (PGPR), leading to significantly greater root growth when compared to the controls. To develop rhizobacterial isolates as biological weed control agents, isolates with 280%

suppression to root growth are selected through this screening process.

Out of 762 rhizobacterizal isolates screened for biological control against downy brome, 4.5% of the population caused a reduction in root growth by 280% (categories O-10 and 1 l-20) (Table 1). When cell-free culture filtrate (CFCF) from rhizobacteria were screened for biocontrol activity against green foxtail, 10.7% (46 out of 430) of the isolates suppressed root growth by 280% (Table 2). When unfiltered supematant (UNF) was used, the success rate for discovering rhizobacteria suppressive to green foxtail increased to 52.5% (105 out of 200) (Table 2). Similarly, use of CFCF resulted in 11.4% (25 out of 220) of the rhizobacterial population suppressive to wild oats by ≥80%, while unfiltered supematant (UNF) resulted in 15.2% (25 out of 164) of the population exhibiting inhibitory effects to wild oat roots (Table 3).

Table 1. Downy brome. Effect of cell-free culture filtrate (CFCF) produced by rhizobacteria on downy brome root growth (% of control).

Root growth1 (% of control)	Number of bacterial isolates	Percent of bacterial population
O-10	29	0.7
1 l-20	55	3.8
21-30	96	7.2
31-40		12.6
41-50	121	15.9
51-60	102	13.4
61-70	98	12.9
71-80	77	10.1
81-90	47	6.2
91-100	46	6.0
101-l 10	28	3.7
111-120	18	2.4
121-130	11	1.4
131-140	13	1.7
141-150	2	1.2
151-160	2	0.3
161-170		0.3
171-180	2	0.3
181-190	1	0.1
Total	762	100.2

1 Number of bacterial isolates and percent of bacterial population demonstrating suppressive properties in various categories (as a percent of control) are presented. Rhizobacteria are inhibitory if root growth is <100 and stimulator-y if root growth is >100.

Effect of nutritional conditions on biocontrol activity

Use of different bacterial culture media to optimize suppression (biological control) of green foxtail growth and development with two rhizobacterial isolates in agar and growth pouch bioassays was investigated. Level of root suppression by isolate GF-10 increased from 74% when grown in minimal medium (PMM) to >80% when grown in nutrient broth (NB) and trypticase soy broth (TSB) (Table 4). The effect was more dramatic for isolate GF-2 where root growth suppression was only 4% in PMM and increased to 90% in NB and 77% in TSB.

Table 2. Green foxtail. Effect of cell-free culture filtrate (CFCF) and unfiltered, centrifuged supematant (UNF) produced by rhizobacteria on green foxtail root growth (% of control).

	CFCF		UNF	
Root growth1 (% of control)	Number of isolates	Percent of population	Number of isolates	Percent of population
O-10	7	1.6	64	32.0
1 l-20	39	9.1	41	20.5
21-30	20	4.7	19	9.5
31-40	40	9.3	8	4.0
41-50	32	7.4	5	2.5
51-60	43	10.0	4	2.0
61-70	50	11.0	15	2.5 7.0
81-90	55	12.8	16	8.0
91-100	36	8.4	12	6.0
101-l 10	20	4.7	7	3.5
111-120	12	2.8	2	1.0
121-130	3	0.7	1	0.5
131-140	3	0.7	2	1.0
141-150	4	0.9	0	0.0
Total	430	100.1	200	100.0

1 Number of bacterial isolates and percent of bacterial population demonstrating suppressive properties in various categories (as a percent of control) are presented. Rhizobacteria are inhibitory if root growth is <100 and stimulatory if root growth is >100.

In growth pouch bioassays (Table 4), both isolates (GF-10 and GF-2) showed increases in their ability to suppress root and shoot growth when cultured in NB and TSB, when compared to PMM. In PMM, suppression of root and shoot growth by GF- 10 was over 74% and 5 1 %, respectively, while root and shoot growth suppression was <10% for isolate GF-2. Suppression of root growth by GF-10 in TSB and NB was >80% and shoot growth suppression was 265%. Suppression of shoot and root growth by isolate GF-2 in NB and TSB was also significantly enhanced, when compared to PMM.

SUMMARY

Several hundred rhizobacterial isolates were screened for their potential as biological control agents of downy brome, green foxtail, and wild oats. Over 100 isolates with 280% suppression to root growth of these weeds in laboratory bioassays have been selected as potential biological control agents. An increase in root growth suppression was exhibited when unfiltered (UNF) bacterial broth was incorporated into agar, compared to CFCF alone. These results suggest that the unfiltered bacterial supematant contains several important components that contribute to the suppression of the weeds and indicate the need for effective and rapid screening methods for research in biological weed control. Moreover, exploration for potential biocontrol agents is an on-going and necessary activity.

Nutritional factors play a role in enhancing biocontrol activity of DRB. Specific requirements in the nutritional environment of the medium and the effect of these changes in terms of propagule yield, efficacy-(biological control activity), and stability will have a significant effect on the potential of a biological control agent and its success as a commercial product.

Table 3. Wild oats. Effect of cell-free culture filtrate (CFCF) and unfiltered, centrifuged supernatant (UNF) produced by rhizobacteria on wild oat root growth (% of control).

	CFCF		UNF	
Root growth1 (% of control)	Number of isolates	Percent of population	Number of isolates	Percent of population
O-10	10	4.5	12	7.3
1 l-20	15	6.8	13	7.9
21-30	21	9.5	31	18.9
31-40	13	7.7	29	17.7
41-50	17	8.1	29	17.7
51-60	18	[illegible]	10	6.1
61-70	17	7.7	13	7.9
71-80	23	10.4	9	5.5
81-90	18	7.7	3	1.8
91-100	17	5.9	6	3.7
101-l 10	13	[illegible]	1	0.6
111-120	16	7.2	5	3.0
121-130	12	5.4	1	0.6
131-140	5	2.3	1	0.6
[illegible]	[illegible]	1.4	1	0.6
141-150	0	0.0	0	0.0
161-170	2	0.9	0	0.0
171-180	1	0.5	0	0.0
Total	221	100.0	164	99.9

1 Number of bacterial isolates and percent of bacterial population demonstrating suppressive properties in various categories (as a percent of control) are presented. Rhizobacteria are inhibitory if root growth is <100 and stimulatory if root growth is >100.

Table 4. Evaluation of bacterial culture media and two rhizobacterial isolates on the suppression of green foxtail root and shoot growth in **agar bioassays** containing cell-free culture filtrate (CFCF) and in **growth pouch bioassays.**

		Agar Bioassay	**Growth Pouch Bioassay**	
Bacterial Isolate	Culture Media	Root Suppression	Root Suppression	Shoot Suppression
GF-10	PMM	74	74	51
	NB	83	81	69
	TSB	81	89	65
GF-2	PMM	4	4	7
	NB	90	77	52
	TSB	77	75	65

PMM = minimal medium, NB = nutrient broth, TSB = trypticase soy broth, GF-10 and GF-2 refer to the rhizobacterial isolates.

ACKNOWLEDGEMENTS

Natural Sciences and Engineering Research Council of Canada, Western Grains Research Foundation, and Saskatchewan Agriculture Development Fund are gratefully acknowledged for providing financial support of this project. Special thanks are extended to K. Sawchyn, T. Bjorgan, S. Blechinger, L. Gibson, K. Hanson, S. Harris, B. Holmstrom-Ruddick, and H. Mooney for their technical assistance.

REFERENCES

Beckie, H.J., and I.N. Morrison. 1993. Effect of ethalfluralin and other herbicides on trifluralin-resistant green foxtail (Setaria viridis). Weed Technol. 7:6-14.

Boyetchko, S.M., and K. Mortensen. 1993. Use of rhizobacteria as biological control agents of downy brome. In: Proceedings of the Soils & Crops Workshop '93, pp. 443448. Saskatoon, Saskatchewan, February 25-26, 1993.

Charudattan, R. 199 1. The mycoherbicide appth plant pathogens. Pages 24-57 *in:* D.O. TeBeest (ed.), Microbial control of weeds, Chapman & Hall Inc., New York.

Charudattan, R., and C.J. DeLoach, Jr. 1988. Management of pathogens and insects for weed control in agroecosystem. p. 245-264. In: M.A. Altieri and M. Liebman (eds.), Weed Management in Agroecosystems: Ecological Approaches. CRC Press, Boca Raton, Florida.

Heap, I.M., B.G. Murray, H.A. Loeppky, and I.N. Monison. 1993. Resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in wil oat *(Avena fatua)*. Weed Sci. 4 1:232-238.

Kennedy, A.C., L.F. Elliott, F.L. Young, and C.L. Douglas. 1991. Rhizobacteria suppressive to the weed downy brome. Soil Sci. Soc. of Amer. J. 55:722-727.

LeBaron, H.M. 1990. Weed science in the 1990s: will it be forward or in reverse? Weed Technol. 4:671-689.

Souissi, T., and R.J. Kremer. 1994. Leafy spurge (Euphorbia esula) cell cultures for screening deleterious rhizobacteria. Weed Sci. 42:310-315.

Swanton, C.J., K.N. Harker, and R.L. Anderson. 1993. Crop losses due to weeds in Canada. Weed Technol. 7:537-542.

TeBeest, D.O., X.B. Yang, and C.R. Cisar. 1992. The status of biological control of weeds with fungal pathogens. Annu. Rev. Phytopathol.