Bacterial Viability and Biological Seed Treatment of Canola

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Introduction

The use of sulfur-oxidizing plant growth promoting rhizobacteria (PGPR) applied as seed treatment for increasing canola yield has been reported previously (Kloepper et al., 1988; Yesmin and Banerjee, 2000). For consistent plant growth response colonization of the rhizosphere and rhizoplane by the PGPR is essential. In many cases, however, results have been variable or not comparable. Inadequate colonization of the roots by the introduced PGPR strain is considered to be a major reason for sub-optimal results (Schippers et al., 1995). Bacterial viability is one of the most important factors for successful and adequate colonization of the rhizosphere and rhizoplane that ultimately affect the plant yield. Thus, the ability of microbial inoculants to successfully colonize expanding root systems is of major importance in determining the potential success of the biological seed treatments (Parke, 1991). Seed is used as a carrier for inoculum and biologicals should be in a state where it can most effectively colonize the emerging roots. Biological activity, however, may decline rapidly between the time of inoculation and seeding to field. The present study investigates the survivals of PGPR on biologically treated seeds with non-coated (bare), peat coated and fungicide-coated seeds. Mixture of PGPR strains were also examined as that might have greater potential to give a consistent performance under different environmental and growth conditions.

Materials and methods

The Microorganisms

Rhizobacterial strains ML1, ML2, ML3, ML6 and MX6 (mixture of PGPRs) were selected on the basis of their S-oxidizing capability and plant growth performance under laboratory and growth chamber conditions. These bacterial strains were isolated from canola crop rhizosphere and were also tested for their ability to oxidize elemental S in the soil environment (Figure 1).

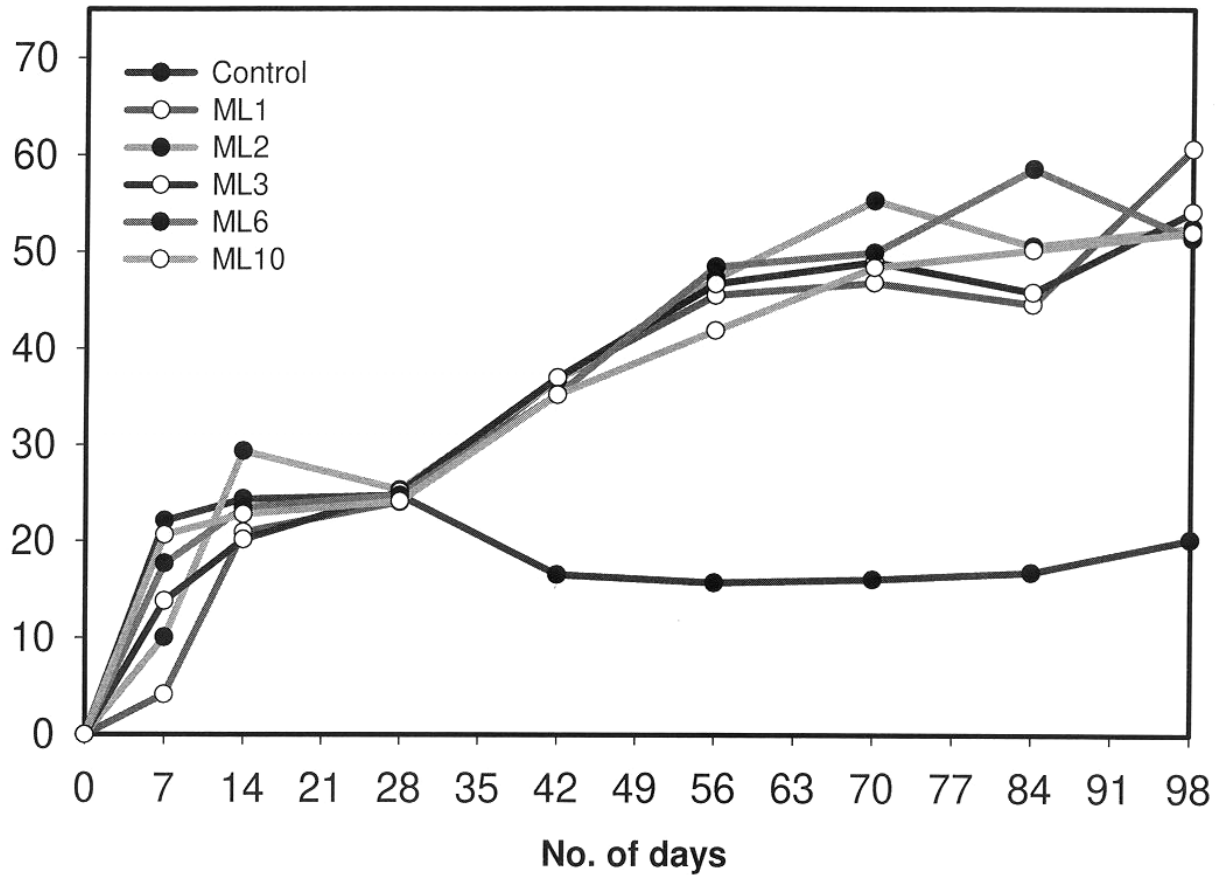

Figure 1. Sulfate production in soil by rhizobacterial strains

Biological Seed Treatment (BST)

The surface sterilized canola seeds were inoculated with isolates ML1, ML2, ML3, ML6 and MX6. Inoculated seeds were dried and kept in sterile jar under room temperature (22°C ± 2°C). A portion of inoculated seed was coated with peat-kaolinite mixture to examine bacterial longevity. To study the compatibility of PGPR strains with Vitavax RS Flowable (a common fungicide used in canola growing areas in the prairies), inoculated seeds were coated with recommended dose (VR) and one half of the recommended dose (VM) of Vitavax. Seeds from control (-BST) and treated seed either bare or coated were sampled at several time intervals up-to 30 days period after inoculation and bacterial population were counted. Biological treated seeds were also used for emergence test in soils from three different locations (Gilbert Plain, MB; Melfort, SK and South Farm, SK) under greenhouse conditions. One-month old control and biologically treated peat coated seeds were germinated in sterile growth pouch to examine the viability of PGPR strains. After 7 days, the shoots (hypocotyl and cotyledon) and roots were macerated separately and bacterial population from the shoots and roots were recorded.

Results

Bacterial survivability in biologically treated canola seeds did not go beyond 10-12 days. For the satisfactory root colonization the desired population level was between 8-10 days (Table 1a). Seed emergence test showed that BST accelerated seed germination significantly within 4 days (Table 2). Surface dried inoculated seeds coated with peat-kaolinite mixture dramatically increased the bacterial survivability onto seeds up-to 28 days compared to the inoculated bare seeds that were up-to 10 days (Tables 1a & 1b). Moreover, the satisfactory colonization by PGPR strains was observed under sterile growth pouch condition in 30 days old stored peat coated seeds (Figure 2). The compatibility study with fungicide Vitavax showed that tested PGPRs were not compatible with the fungicide at recommended dose and seed emergence was drastically reduced except MX6 treatment (Table 3). However, better seed emergence was shown when lower rate of Vitavax was used with the PGPRs (Table 3).

Table 1a. Viability of bacteria after bacterial inoculation of canola seeds.

Treatment	Bacterial population in biologically treated seeds (cfu X 10^5)								
	Day 2	Day 4	Day 7	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18
Control	0	0	0	0	0	0	0	0	0
Control + ML1	157	115	48	30	7	3	0	0	0
Control + ML2	142	85	20	16	11	1	0	0	0
Control + ML3	96	81	52	37	27	14	6	0	0
Control + ML6	215	112	63	51	20	0	0	0	0
Control + VR	0	0	0	0	0	0	0	0	0
Control + VR + ML1	6	1	0	0	0	0	0	0	0
Control + VR + ML2	16	2	0	0	0	0	0	0	0
Control + VR + ML3	13	7	2	0	0	0	0	0	0
Control + VR + ML6	10	4	1	0	0	0	0	0	0
Control + VR + MX6	23	14	5	1	0	0	0	0	0

Note: ML denotes bacterial isolate; MX denotes bacterial mixture.

Table 1b. Bacterial count in biologically treated canola seeds coated with peat.

Treatment	Bacterial population in biologically treated seeds coated with peat (cfu X 10^5)								
	Day 2	Day 4	Day 8	Day 10	Day 14	Day 18	Day 21	Day 28	Day 30
Control	0	0	0	0	0	0	0	0	0
Control + ML1	155	116	57	22	12	4	2	1	0
Control + ML2	146	92	35	20	9	7	1	0	0
Control + ML3	104	84	66	44	28	19	7	2	1
Control + ML6	213	125	67	30	12	4	1	0	0
Control + MX6	208	109	80	52	30	22	15	8	2

Note: ML denotes bacterial isolate; MX denotes bacterial mixture.

Table 2a. Canola seed emergence after bacterial inoculation in Gilbert Plain soil.

Bacterial Isolates	Seed Germination (%)				
	2 day	4 day	6 day	8 day	10 day
Control	0	30	73	90	90
ML1	0	60	87	93	93
ML2	0	63	80	97	97
ML3	0	73	97	100	100
ML6	0	60	87	97	97

Table 2b. Canola seed emergence after bacterial inoculation in Melfort soil.

Bacterial Isolates	Seed Germination (%)				
	2 day	4 day	6 day	8 day	10 day
Control	0	23	70	87	90
ML1	0	47	77	87	90
ML2	0	53	73	90	90
ML3	0	57	87	90	90
ML6	0	53	77	90	93

Table 2c. Canola seed emergence after bacterial inoculation in South Farm soil.

Bacterial Isolates	Seed Germination (%)				
	2 day	4 day	6 day	8 day	10 day
Control	0	20	77	87	87
ML1	0	40	73	80	87
ML2	0	47	77	87	90
ML3	0	47	87	97	97
ML6	0	47	77	87	90

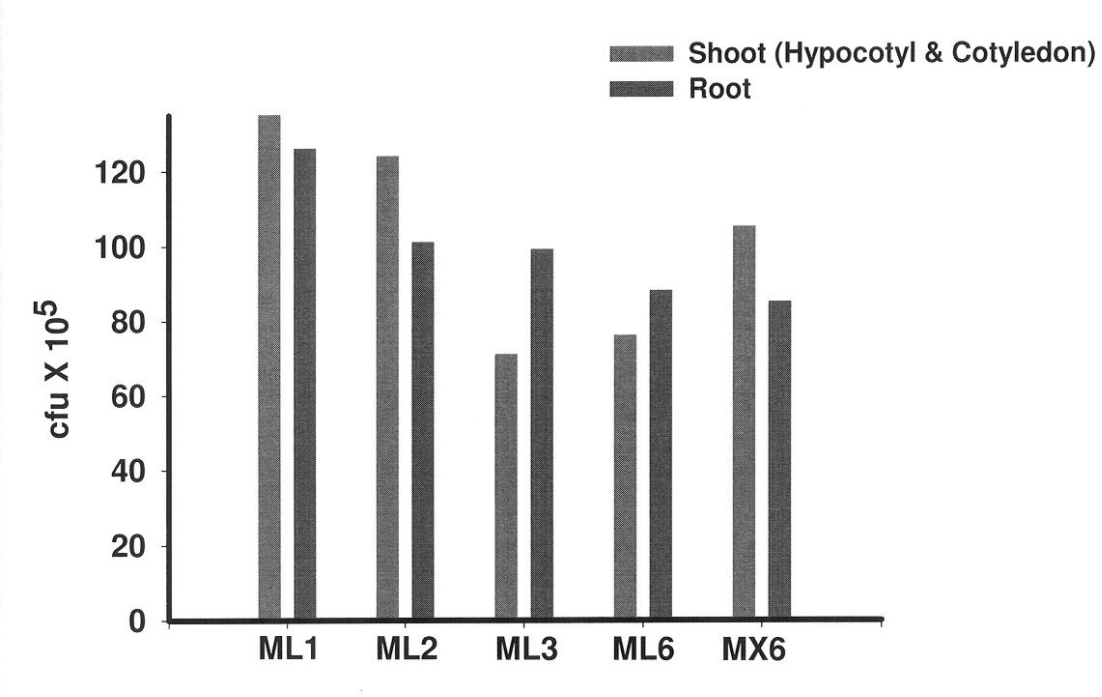

Figure 2. Bacterial count in seedlings from one month old biologically treated seed

Table 3. Canola seed emergence test in Gilbert Plain soil.

Treatment	Seed emergence soil (%)				
	Day 2	Day 4	Day 6	Day 8	Day 10
Control	0	37	80	93	93
Control + ML1	0	63	90	97	97
Control + ML2	0	60	87	97	97
Control + ML3	0	67	97	100	100
Control + ML6	0	57	87	97	97
Control + MX6	0	80	97	100	100
Control + VR	0	33	76	90	90
Control + VR + ML1	0	27	57	67	67
Control + VR + ML2	0	23	50	57	60
Control + VR + ML3	0	27	57	70	70
Control + VR + ML6	0	17	43	50	50
Control + VR + MX6	0	33	73	80	80
Control + VM	0	36	80	93	93
Control + VM + ML1	0	36	80	90	90
Control + VM + ML2	0	33	77	90	90
Control + VM + ML3	0	37	83	93	93
Control + VM + ML6	0	33	83	90	90
Control + VM + MX6	0	50	83	97	100

Note: ML denotes bacterial isolate; MX denotes bacterial mixture.

Conclusion

Drastic decline in bacterial population was observed when biologically treated seeds were coated with common fungicide Vitavax.

The technology of coating biologically treated seeds can offer possibilities of optimizing the survivals and functioning of the PGPRs.

Bacterial mixture (consortia) showed opportunity that could give a more consistent performance under different agro-climatic conditions. Nevertheless, more impact study of these PGPRs on crop cultivars and soil types are needed.

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