Comparison of Inoculant Media for Sulfur-Oxidizing Plant Growth Promoting Rhizobacteria in Canola

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Introduction

The ability of rhizosphere microorganisms to influence plant growth is gaining considerable attention worldwide. Various studies have already proven the beneficial effect of plant growth promoting rhizobacteria (PGPR) on different agricultural crops (Kloepper, 1993; Banerjee, 1995). Canola, like other oil seed crop has high sulfur demand. In recent years, several attempts have been made to utilize S-oxidizing microorganisms to meet plant S requirement and to substitute costly S fertilizer. But the success is variable, as the information on S-oxidizing PGPR is limited and the mechanisms also not yet fully understood. In many cases, no standard methods were followed and different inoculation media were used. The population density and activities of microorganisms could vary with the inoculation media. The aim of the present study was to compare the effects of PGPR if any due to the difference in the commonly used inoculant media like seed, soil and elemental S fertilizer.

Bacteria Selection

The 465 presumptive S-oxidizing rhizobacteria were isolated from elemental S enrichment media. All of these bacteria were tested qualitatively for their ability to oxidize S^0 and $S_2O_3^{2-}$ *in vitro* (Grayston and Germida, 1991). For quantitative test, an incubation study was performed with bacteria that showed positive results in the qualitative test. The incubation was done at 28^0C with known amount of sterile S^0 (flowable S - Stoller Chemical Co., Inc., Houston). Sulfate production were measured at 7, 14, 28, 42 and 56 days of incubation following Banerjee and Dey (1992). Bacterial S-oxidizing capabilities are presented in Figure 1.

Selected rhizobacterial strains were tested for canola (*Brassica napus L.* var Agassiz) seed germination on petri plates under laboratory conditions (Table 1). Seed inoculation accelerated canola seed germination.

Three of six superior strains were tested for their survivability in canola seeds. Control and inoculated seeds were germinated in sterile growth pouch containing different nutrient solutions.

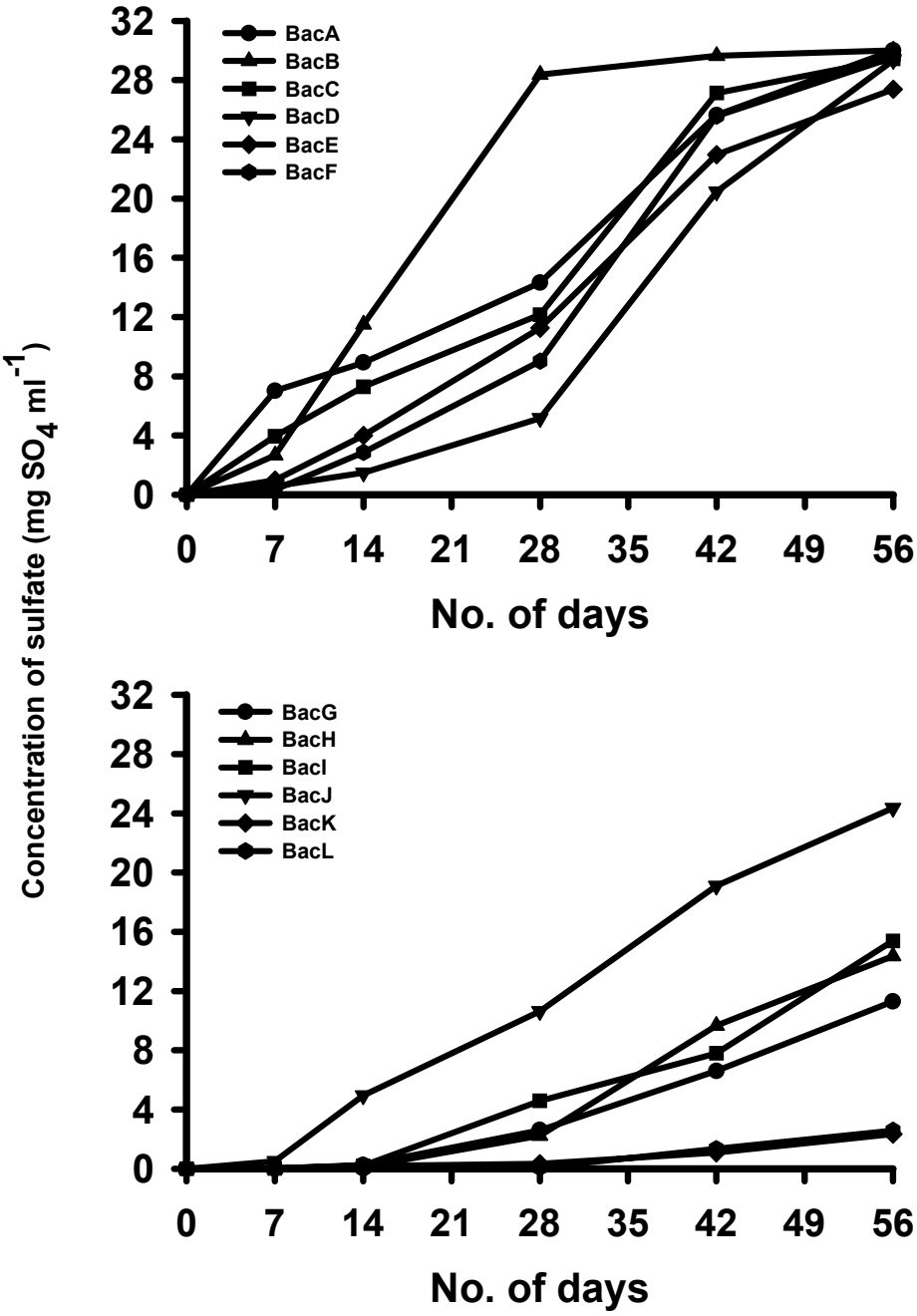

Figure1. Sulfate production by rhizobacterial strains

Table 1. Canola seed germination after bacterial inoculation.

Bacterial Isolates	Seed germination (%)				
	2 day	4 day	6 day	8 day	10 day
Control	47	70	90	96	100
*BacA	80	90	100	100	100
BacB	80	98	98	100	100
BacC	80	100	100	100	100
BacD	77	80	98	98	98
BacE	70	80	96	96	96
BacF	75	80	98	100	100
BacG	60	90	98	98	98
BacH	60	90	96	98	98
BacI	70	90	98	100	100
BacJ	75	98	98	100	100
BacK	75	95	98	100	100
BacL	60	80	95	95	95

*Bac denotes bacterial isolate

The shoots (hypocotyl and cotyledon) and roots were macerated separately and spread on TSA plates to obtain inoculated bacteria. Plates from shoots and roots of all inoculated plants resulted numerous bacterial colonies but none were observed from plates of control plants.

Three selected bacteria (BacA, BacC and BacJ) were used for inoculantion media comparison study.

Greenhouse Experiment

The canola was grown in sterilized and non-sterilized soil. Soil was collected from South Farm, Swift Current and the soil characteristics are presented in Table 2. The inoculant media used were: seed, soil and elemental S fertilizer (Tiger-90 and precipitated elemental S).

Soil sterilization expedited the plant maturation period along with early flowering and pod formation. Soil sterilization had significant effect on the plant growth. Therefore, the results for sterile and non-sterile soils were analyzed and presented separately in Table 3 and Table 4. For sterilized soil, specific bacterial activity in each media was compared with controls (Table 3a). The bacterial inoculation increased canola biomass and seed yield regardless of media. When the data was pooled for media comparison (Table 3b), differences in inoculation media showed significant effect on canola stalk dry weight ($p<0.05$) and seed weight ($p<0.10$).

For non-sterilized soil, specific bacterial activity in each media was also compared with controls (Table 4a). Like sterile soil, bacterial inoculation increased canola biomass and yield regardless of media. When the data was pooled for media comparison (Table 4b), inoculation media showed significant effect on canola stalk dry weight ($p<0.10$), pod number ($p<0.10$) and seed weight ($p<0.10$).

Table 2. General characteristics of the soil used in the study.

Texture		**Sandy loam**
	Sand (%)	**58.5**
	Silt (%)	**25**
	Clay (%)	**16.5**
pH	**in H_2O**	**6.84**
EC (dS m^{-1})	**in saturated paste**	**1.01**
N (μg g^{-1})	**$NaHCO_3$-extractable NO_3-N**	**49.9**
P (μg g^{-1})	**$NaHCO_3$-extractable PO_4-P**	**23.07**
K (μg g^{-1})	**NH_4OAC-extractable K**	**203**
S (μg g^{-1})	**$CaCl_2$-extractable SO_4-S**	**7.21**
Ca (μg g^{-1})	**NH_4OAC-extractable Ca**	**1094**
Mg (μg g^{-1})	**NH_4OAC-extractable Mg**	**205.8**
Na (μg g^{-1})	**NH_4OAC-extractable Na**	**11.25**

Conclusion

- **Seed inoculation consistently showed positive effect on canola biomass and seed yield compared to soil or fertilizer inoculation.**
- **Seed inoculation also accelerated canola seed germination.**
- **Bacteria in soil or fertilizer inoculation have high mobility with limited soil volume in pot experiment. While in field situation, bacteria in soil or fertilizer inoculation might need time to disperse within the soil before come in close contact with the growing roots to make an impact on plant growth promotion.**

References

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Table 3a. Effect of inoculation media on biomass and yield of canola inoculated with different rhizobacterial strains grown in sterilized soil in the green house.

Inoculation media	Bacterial code	Stalk dry wt. (g/plant)	Pod no. (no./plant)	Seed wt. (g/plant)
Seed	BacA	6.33	161	4.62
	BacC	5.76	154	4.47
	BacJ	5.54	145	4.24
Soil	BacA	5.07	144	4.22
	BacC	4.64	139	4.13
	BacJ	3.85	132	3.74
PES	BacA	4.30	134	4.09
	BacC	4.39	137	4.12
	BacJ	4.16	134	4.02
T-90	BacA	4.73	143	4.32
	BacC	4.78	139	4.24
	BacJ	4.29	132	4.04
Control + PES		4.15	125	2.92
Control + T-90		4.12	121	2.80
Comparing each bacteria as separate treatment including control				
LSD 10%		1.78	24.23	0.47
5%		2.14	29.08	0.56
1%		2.86	38.89	0.75

Table 3b.

Inoculation media	Stalk dry wt. (g/plant)	Pod no. (no./plant)	Seed wt. (g/plant)
Seed	5.88	153	4.44
Soil	4.52	138	4.03
PES	4.28	135	4.08
T-90	4.60	138	4.20
LSD 10%	0.97	13.75	0.27
5%	1.16	16.49	0.33
1%	1.55	22.03	0.44

Table 4a. **Effect of inoculation media on biomass and yield of canola inoculated with different rhizobacterial strains grown in non-sterilized soil in the green house.**

Inoculation media	Bacterial code	Stalk dry wt. (g/plant)	Pod no. (no./plant)	Seed wt. (g/plant)
Seed	BacA	5.25	111	3.43
	BacC	5.44	115	3.55
	BacJ	5	107	3.32
Soil	BacA	4.91	99	3.08
	BacC	4.09	105	3.26
	BacJ	4.62	102	3.24
PES	BacA	4.59	112	3.36
	BacC	4.22	100	3.39
	BacJ	4.52	106	3.23
T-90	BacA	4.32	103	3.05
	BacC	5.01	98	2.59
	BacJ	4.3	103	3.14
Control + PES		3.72	101	2.51
Control + T-90		4.06	97	2.32
Comparing each bacteria as separate treatment including control				
LSD 10%		0.99	14.66	0.59
5%		1.19	17.59	0.70
1%		1.58	23.52	0.94

Table 4b.

Inoculation media	Stalk dry wt. (g/plant)	Pod no. (no./plant)	Seed wt. (g/plant)
Seed	5.23	111	3.43
Soil	4.54	102	3.19
PES	4.44	106	3.33
T-90	4.54	101	2.93
LSD 10%	0.55	7.45	0.32
5%	0.66	8.94	0.39
1%	0.89	11.94	0.52