Temperature, pH and media requirements for bacterial antagonism of soilborne pathogens

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ABSTRACT

Select rhizobacteria, *Pseudomona* fluorescens strain 63-49 and *P. cepacia* strain Ral-3, were evaluated for their ability to suppress the growth of several isolates of *Rhizoctonia solani* and *Cochliobolus sativus,* respectively, in liquid medium, on several solid media, at different temperature regimes and at varying pH. The two bacterial strains tested significantly reduced the growth of the fungi, irrespective of the growth medium, temperature or pH. Among the solid media tested, the efficacy of bacteria against fungi was best on Potato Dextrose Agar (PDA) for strain 63-49 and Pseudomonas Agar F (PAF) for strain Ral-3. However, liquid medium results were quite variable depending on the fungal isolate especially for C. *sativus.* The activity of strain 63-49 was highest at 22°C and the acitvity of strain Ral-3 was at 30°C.

INTRODUCTION

Control of plant pathogenic organisms by xenobiotic compounds is increasingly falling out of favor. The use of biological control agents (BCAs) is becoming more attractive due to environmental and economic reasons. In the past several years, there have been numerous reports of rhizobacteria suppression diseases caused by soilbome plant pathogens. The suppression of the plant pathogens has been associated with bacterial antagonism which may be influenced by several biotic and abiotic factors. This research was conducted to determine the influence of abiotic factors such as culture media, temperature and pH on the level of antagonism.

MATERIALS AND METHODS

Source of bacterial and fungal isolates

Two rhizobacterial strains 63-49 *(Pseudomonas fluorescens)* and Ral-3 *(P. cepacia)* were selected from the Cominco Fertilizer Ag Biologicals culture collection. These strains were chosen because of their previous activities. Strain 63-49 has demonstrated significant improvement of healthy stand and gram yield of canola grown in *Rhizoctonia solani* infested fields and strain Ral-3 has significantly suppressed common root rot of wheat caused by *Cochliobolus sativus.* Bacterial cultures were stored at -80°C in tryptic soy broth (TSB) amended with 20% glycerol prior to use. The rhizobacteria were produced in proprietary commercial formulation and stored in commercial packages for use in experiments.

Eight isolates of *R. solani* and C. *sativus* obtained from different sources, as shown in Table 1, were selected for use in this study.

Table 1. Source of fungal isolates used in this study.

Fungal Pathogen	Isolate	Source
Rhizoctonia solani	RN3	Canola roots, Winnepeg, MB
	RN10	Canola roots, Winnepeg, MB
	US-Standard	Unknown
	I4	Canola roots, Melfort, SK
	5259	Canola roots, Cutknife, SK
	P32-02	Canola roots, Edmonton, AB
	65M1	Canola roots, Tway, SK
	68M9	Canola roots, Tway, SK
Cochliobolus sativus	Tl-120	Wheat internode, Regina,SK
	Wl-120	Barley internode, Landis, SK
	1133	Wheat internode, Saskatoon, SK
	91-11	Wheat internode, Saskatoon, SK
	CAWI	Wheat root, Lethbridge, AB
	CAB1	Barley root, L&bridge, AB
	RO02	Barley root, North Dakota, USA
	ND183	Wheat root, North Dakota, USA

Influence of biotic factors on antibiosis of Pseudomonads

As shown in Table 2, the level of antagonism of strain 63-49 on eight *R. **solani*** isolates and strain Ral-3 on eight C. sativus isolates were tested in one liquid medium, on five different solid media, at six different temperatures and five different pH levels. The media were prepared and autoclaved 121°C for 15-20 minutes. Adjustment of the pH of the media was carried out by varying amounts of KH_2PO4 and K2HP0$ for PAP prior to sterilization and by aseptically adding varying amounts of IN HC1 and 1N NaOH to PDA after autoclaving.

Table 2. Growth factors used.

Solid Media	Liquid Medium	Temperature	p	H
		(°C)	on PDA	on PAF
Potato Dextrose Agar (PDA)	Trypticase Soy Broth (TSB)	5	4.5	6.0
Pseudomonas Agar F (PAF)		15	5.0	6.5
Corn Meal Agar (CMA)		22	5.6	7.0
Yeast Extract Agar (YEA)		30	6.0	7.5
Malt Extract Agar (MEA)		35	6.5	7.9
		40		

Plugs of mycelium (5 mm diameter) were cut from the edge of an actively growing fungal colony on PDA and one plug was inverted and placed in the centre of each test plate or dropped into the 50 ml of liquid medium. Two parallel streaks of rhizobacteria (3.5 cm long) were placed 3 cm apart on either side of the mycelial plug on solid media or 100 µl of rhizobacteria was added to the liquid medium. Plates were incubated in the dark for 4-10 days and liquid cultures in the dark with agitation for 2-3 weeks. On the solid media, colony diameter was measured and compared to that of colonies from mycelial plugs not challenged by rhizobacteria. Dry weights of fungal mycelium recovered from the liquid by filtration were compared to that of mycelium from not challenged by rhizobacteria. All tests for each rhizobacteria and each pathogen isolate combination were carried out in triplicate. The data was analyzed using ANOVA and the mean values seperated by Fisher's Protected LSD at $p=0.05$. Results were presented as proportion suppression to that of the control (nonbacterized treatment).

RESULTS

The suppression of the radial growth of the eight *R. solani* isolates by strain 63-49 on the various solid media was significant (Figure 1). The suppression by strain 63-49 on the eight isolates was grouped into 3 patterns (Figure 1). From the analyzed data, it was determined that the best suppression of *R. solani* by 63-49 was on PDA. Similarly, the suppression of the radial growth of C. *sativus* isolates was significant and was grouped into 3 patterns (Figure 2). Analysis showed that suppression of C. *sutivus* by Ral-3 was found to be significantly better on PAF compared to the other media. These results indicate that the rhizobacteria have the ability to suppress a variety of fungal isolates under a wide range of nutritional conditions and that media does affect the level of suppression.

Figure 1. Suppression of R. solani by strain 63-49 on various solid media.

Figure 2. Suppression of C. sativus by strain Ral-3 on various solid media.

{---See end of paper for figures---}

In liquid culture, the suppression of *R. solani* isolates by strain 63-49 was all very similar (Figure 3). Suppression of C. *sutivus* by Ral-3 in liquid medium was quite varied (Figure 3). The use of liquid culture was determined to be too variable for use in subsequent growth factor studies.

Figure 2. Suppression of fungal isolates in liquid medium.

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The suppression of the fungal isolates was found at temperatures ranging from 15°C to 35°C but the degree of suppression was determined to be dependent on the temperature of incubation in both cases as shown in Tables 3 and 4. Fungal growth was not observed at 5°C and 40°C after 4 days incubation for *R. solani. No* visible growth of 63-49 at 40°C was observed, as well. Maximum mean suppression of *R. solani* by strain 63-49 was at room temperature (22°C). No fungal growth was observed after 6 days incubation for C. *sativus* Suppression by strain Ral-3 of C. *sativus was* greatest at **30°C.**

Table 3. Suppression of R. solani by strain 63-49 at different temperatures.

			Temperatures (°C)			
Isolate	**5**	**15**	**22**	**30**	**35**	**40**
RN3	NFG	0.74	0.74	0.70	0.46	NFG
RN10	NFG	0.70	0.78	0.64	0.11	NFG
P32-02	NFG	0.81	0.83	0.51	0.14	NFG
65M1	NFG	0.69	0.74	0.74	0.18	NFG
5259	NFG	0.72	0.73	0.71	0.12	NFG
68M9	NFG	0.67	0.68	0.32	0.17	NFG
US-Standard	NFG	0.73	0.75	0.71	0.07	NFG
I4	NFG	0.68	0.71	0.67	0.00	NFG
Mean	-	**0.72 a**	**0.74 a**	**0.62 b**	**0.15 c**	-

NFG = No Fungal Growth
Mean values with different letters are significantly different based on Fisher's protected LSD.

Table 4. Suppression of C. sativus by strain Ral-3 at different temperatures.

			Temperatures (°C)			
Isolate	**5**	**15**	**22**	**30**	**35**	**40**
1133	NFG	0.60	0.80	0.84	0.56	NFG
W1-120	NFG	0.61	0.79	0.81	0.32	NFG
CAWI	NFG	0.69	0.70	0.81	0.54	NFG
CABI	NFG	0.73	0.71	0.88	0.55	NFG
T1-120	NFG	0.80	0.87	0.87	0.43	NFG
R002	NFG	0.82	0.87	0.89	0.64	NFG
ND183	NFG	0.83	0.85	0.83	0.57	NFG
91-11	NFG	0.88	0.82	0.87	0.49	NFG
Mean	-	**0.75 b**	**0.80 ab**	**0.85 a**	**0.51 c**	-

NFG = No Fungal Growth
Mean values with different letters are significantly different based on Fisher's protected LSD.

The suppression of *R. solani* by strain 63-49 was found to be best at pH 6.0 as shown in Table 5. This was not significantly different from pH 5.6 or 5.0 but was different from pH 6.5. C. *sativus* was suppressed by strain Ral-3 equally well at pH 6.0 and 6.5 as shown in Table 6. Suppression at those pH levels was significantly better than that observed on pH levels of 7.0 and above.

Table 5. Suppression of R. solani by strain 63-49 at different pH levels.

Strain	pH 4.5	oH 5.0	pH 5.6	pH 6.0	pH 6.5
RN3	NBG	0.56	0.49	0.50	0.41
RN10	NBG	0.62	0.62	0.62	0.64
US-Standard	NBG	0.70	0.69	0.71	0.69
14	NBG	0.63	0.59	0.69	0.66
5259	NBG	0.58	0.61	0.62	0.59
P32-02	NBG	0.62	0.62	0.64	0.59
65M1	NBG	0.62	0.61	0.64	0.62
68M9	NBG	0.64	0.63	0.64	0.59
Mean		**0.62 a b**	**0.61 a b**	**0.63 a**	**0.60 b**

NBG = No Bacterial Growth
Mean values with different letters are significantly different based on Fisher's protected LSD.

Table 6. Suppression of C. sativus by strain Ral-3 at different pH levels.

Strain	pH 6.0	pH 6.5	pH 7.0	pH 7.5	pH 7.9
T1-120	0.77	0.77	0.72	0.76	0.74
W1-120	0.75	0.74	0.74	0.76	0.75
1133	0.79	0.82	0.76	0.75	0.74
91-11	0.74	0.75	0.69	0.73	0.71
CAWI	0.79	0.75	0.72	0.75	0.74
CABI	0.75	0.76	0.72	0.65	0.64
R002	0.77	0.79	0.74	0.72	0.73
ND183	**0.78**	**0.76**	**0.75**	**0.76**	**0.79**
Mean	**0.77 a**	**0.77 a**	**0.73 b**	**0.74 b**	**0.73 b**

Mean values with different letters are significantly different based on Fisher's protected LSD.

CONCLUSIONS

For strain 63-49's suppression of R. ***solani*** it was found:

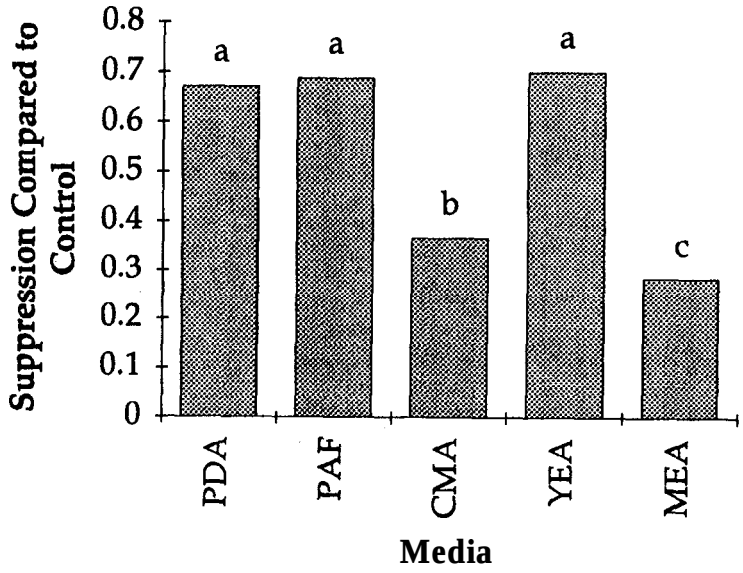
1) although there was variation, radial growth was suppressed on all solid media tested,
2) PDA gave the best and most consistent suppression,
3) suppression in liquid medium (TSB) was varied,
4) temperature affected the level of suppression with 22°C being highly significant compared to 35°C,
5) pH affected suppression with media of pH 6.0 giving significantly better suppression than pH 6.5.

For strain Ral-3's suppression of C. sativus it was found:

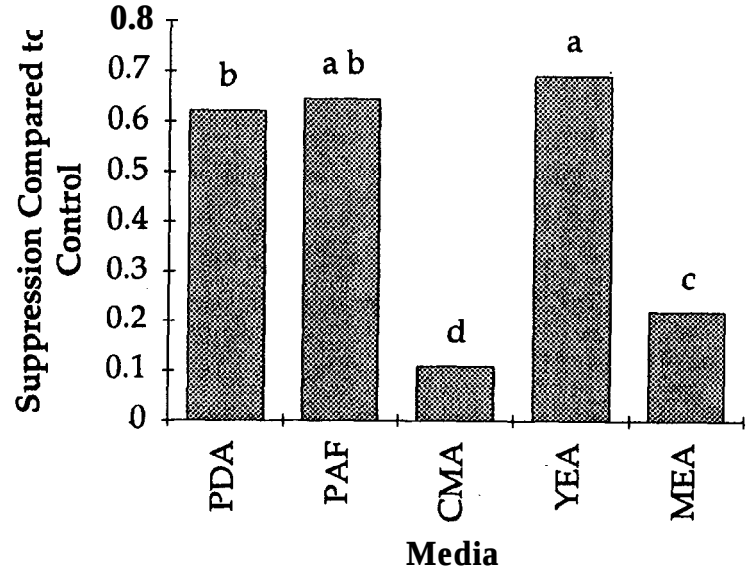
1) radial growth varied on solid media but suppression was found on all solid media tested
2) PAF was significantly better than the other solid media
3) suppression in liquid medium (TSB) was varied
4) temperature affected the level of suppression with 30°C giving the most and 35°C giving the least
5) Ral-3 did not grow on media with pH of 4.5
6) pH affected suppression with media of pH 6.0 and 6.5 giving significantly better suppression compared to the other pH levels.

Figure 1. Suppression of R. solani by strain 63-49 on various solid media.

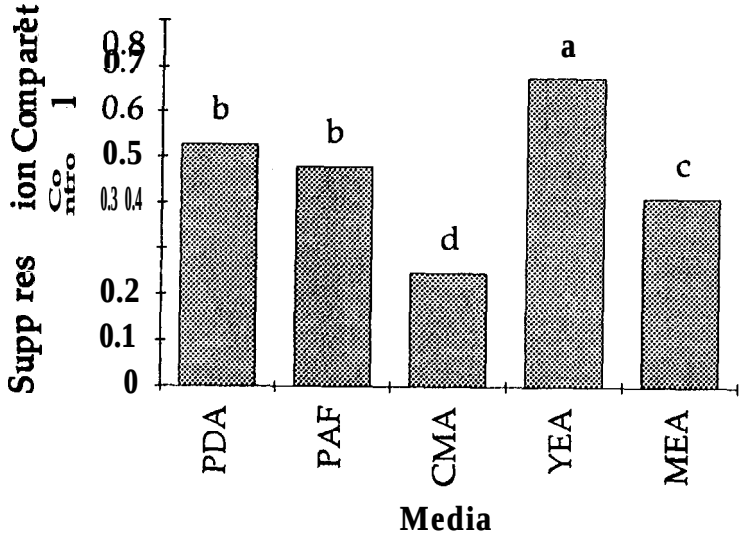
a) **Group A - 14**



b) **Group B - 68M9**



c) **Group C - RN3**

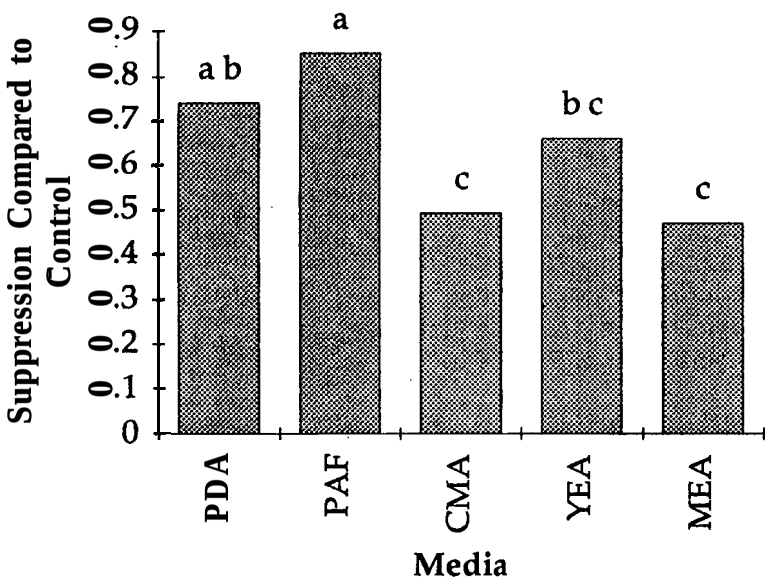


a) Group A consists of 6 of the 8 isolates tested which all gave very similar responses. b) Group B consists of only isolate 68M9 and shows very poor suppression on CMA. c) Group C consists of only isolate RN3 and shows poor suppression on PDA and PAF.

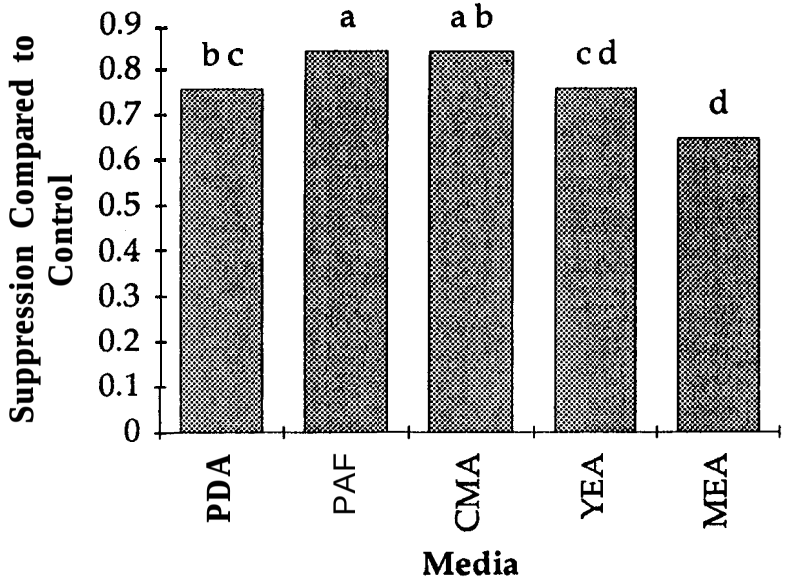
Within graphs, columns with the same letter are not significantly different at p = 0.05 according to Fisher's protected LSD test.

Figure 2. Suppression of C. sativus by strain Ral-3 on various solid media.

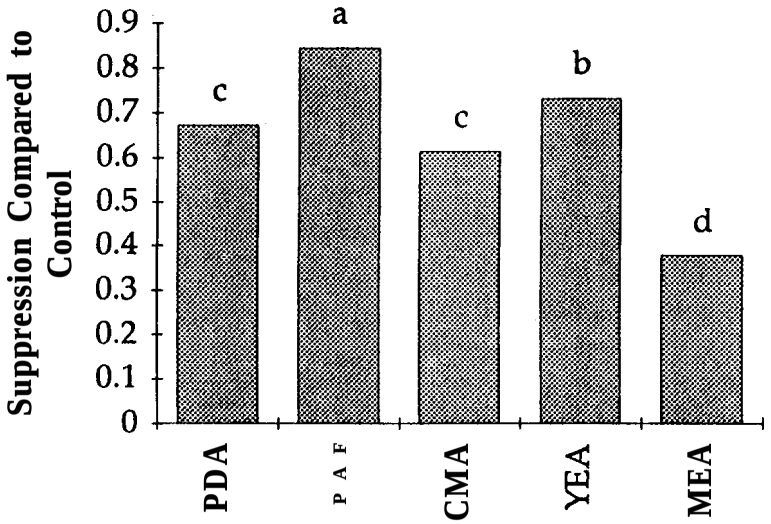
a) Group A - CAWI



b) Group B - ND183



c) Group C - W1 12₀



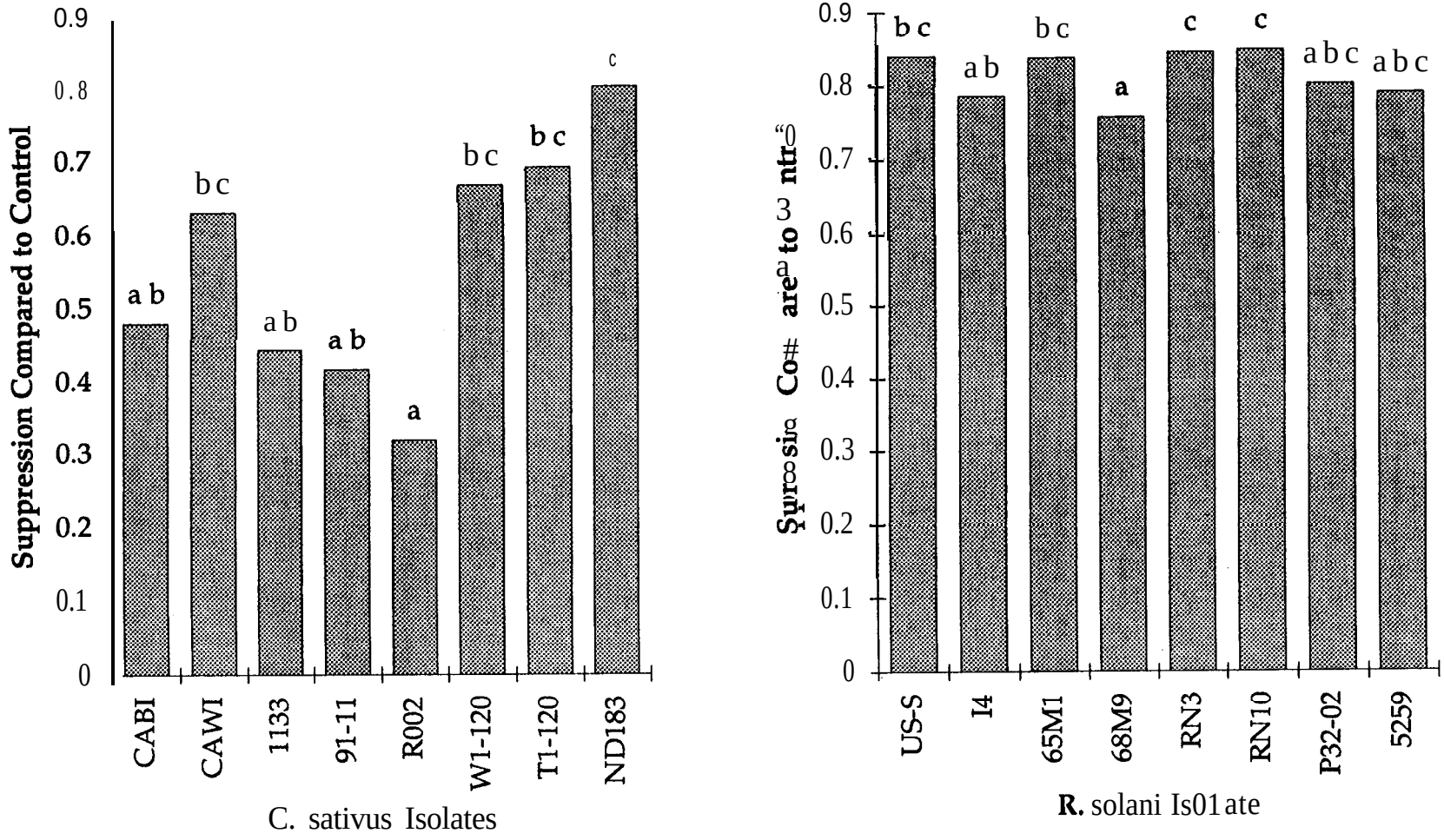
a) Group A consists of 6 of the 8 isolates tested which all gave very similar responses. This group is represented by isolate CAWI. b) Group B consists of only isolate ND183 which shows increased suppression on CMA compared to the other isolates. c) Group C consists only of isolate W1-120 which shows decreased suppression on PDA.

Within graphs, columns with the same letter are not significantly different at p = 0.05 according to Fisher's protected LSD test.

Figure 3. Suppression of fungal isolates in liquid medium.

a) By strain Ral-3

b) By strain 63-49



Columns with the same letter are not significantly different at p=0.05 based on Fisher's protected LSD.