Science and Cultivation of Edible Fungi, Van Griensven (ed.)
© 2000 Balkema, Rotterdam, ISBN 90 5809 1430

Siderophore producing bacteria as potential biocontrol agents of mushroom diseases

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ABSTRACT : Out of 50 isolates of fluorescent pseudomonads isolated from casing mixtures, 34 produced siderophore. After a preliminary screening six siderophore producers and a non siderophore producer were selected to study their biocontrol potential. In *in vitro* dual culture tests against eight parasitic fungi affecting mushroom, isolate Clc reduced growth of *Sepedonium* and *Verticillium* sp. Isolate Cllb highly antagonistic against all the pathogens except *Cladobotryum* sp., *Fusarium* sp. *Neurospora* sp. and *Verticillium* sp.significantly however, isolate CVIa effectively suppressed the growth of *Sepedonium* sp. *Fusarium* sp. and *Mycogone* sp. Isolate Clllb, CIVa and CHId were almost ineffective against most of the pathogens.

In order to outline the potential of bacterial isolates in control of pathogens in mushroom beds the isolates found in *in vitro* tests were used further in casing. The isolates Clc and Cllb worked very well in control of *Sepedonium* sp. in mushroom beds, while significantly higher yield was recorded by using isolate Cllb and CVIa against *Fusarium* sp. The pathogens *Neurospora* sp., *Trichoderma* sp., *Verticillium* sp., *Mycogone* sp., were best controlled by isolates Cllb. The paper reveals that the siderophore producing bacteria isolated from casing are useful in controlling the pathogenic fungi *ofAgaricus bisporus.*

1 INTRODUCTION

A number of bacterial species have so far been tried as biocontrol agents (Weller, 1988). Mechanisms by which bacteria exhibit biological control include antibiosis, competition, parasitism and production of plant growth promoting compounds (Kloepper, 1992). Fluorescent pseudomonads exhibit mechanisms of suppression of pathogens, such as siderophore mediated suppression, production of volatile compounds (HCN, antibiotics mediated suppression, successful colonization and induced resistance (O'Sullivan & O'Gara. 1992). Jhune et al. (1990) have studied biological control of *Mycogone perniciosa* parasitizing *A. bisporus.* Some of the bacteria isolated from biofertilizers have exhibited a strong antagonism against the common competitor mould (Ahlawat & Rai, 1997).

In the light of the above, experiments were conducted to explore and exploit the potential of siderophore producing pseudomonads naturally occurring in casing mixtures in controlling fungi pathogenic on *A. bisporus.*

2 MATERIALS AND METHODS

2.1 *Isolation ofbacterial biocontrol agents*

Employing serial dilution plate technique (Waksman, 1922), total bacterial and fluorescent pseudomonads were isolated from casing mixtures on soil extract medium (Allen, 1957) and

modified King's B medium (King et al., 1954, Sands & Rovira, 1970), respectively.

2.2 *Isolation and identification of pathogens*

The pathogens *(Sepedonium chrysosporium, Neurospora crassa, Mycogone perniciosa, Verticillium fungicola, Trichoderma harzianum, Cladobotryum dendroides* and *Fusarium moniliforme)* were isolated from compost/casing/fruit bodies showing symptoms of disease on peptone-dextrose -rose bengal agar medium (Martin, 1950).

The cultures of the above pathogens were purified and then their identity was confirmed comparing their morphological characteristics with authentic literature (Fletcher et al. 1986; Sharma, 1995). Subsequently, purified cultures were maintained on potato-dextrose-agar (PDA) or compost decoction agar (CDA) slants and subsequently multiplied on PDA and CDA medium.

2.3 *Screening ofsideropboreproduction*

To assess the siderophore producing potential of the bacterial flora isolated universal chrome azurol assay (Schwyn & Neilands, 1987) and iron deficient (Meyer & Abdullah, 1978) were used.

2.4 *Screening of the antagonists*

The antagonism between selected bacterial isolates and the pathogenic fungi were tested following the method suggested by Morton & Stroube (1955). 20.0 ml of sterilized and melted PDA was poured in 90 mm sterilized petri plates. After solidification the plates were inoculated with test fungus using 5 mm diameter disc of young culture, thereafter, a 5 mm diameter filter paper disc dipped in bacterial suspension of individual isolates was placed at a distance of 10 mm from the periphery of inoculated plates. The plates were incubated at 25° C for 10 days and the observations were recorded. Each treatment was replicated thrice.

2.5 *Interaction ofantagonistice isolaties andpathogens in mushroom beds*

To assess the biocontrol potential of bacterial isolates in beds, four isolates CIc Cllb, CVa and CVIa were selected on the basis of *in vitro* test against selected pathogens of *A. bisporus.* Selected isolaties were then mass multiplied on nutrient broth and two different treatments were given in the casing soil at two different doses. In the first set of experiments, bacterial broth containing 10^6 CPU per ml was sprayed at two doses viz., 100 and 200 ml on casing already inoculated with pathogen prepared on wheat grains at 0.75% by weight, then 3.5 cm thick casing was applied over the full spawn run compost in polythene bags. Two more sprays were given at 4 days interval using the same dose.

In the second set, 300 ml and 600 ml bacterial broth were mixed in casing at the time of casing application. Two different checks were also run simultaneously.

3 RESULTS AND DISCUSSION

Biological control with bacteria particularly pseudomonads holds great promise for controlling soil borne diseases caused by fungi (Welier, 1988). Alike field crops, mushrooms suffers due to parasitization by several fungal pathogens. Since pseudomonads constitute a sizeable part of microbial populations of casing microflora and many of them produce siderophores which improve plant growth and also reduce parasitic activities (Byer & Sikora, 1990).

The results recorded are presented in Table 1 and 2 and Fig. 1, 2 and 3. Six isolates of fluorescent pseudomonads tested were siderophore producers while one was non-siderophore producer. Of the seven isolates, isolate CIc Cllb and CVa. reduced the radial growth of *Sepedonium* sp., causing yellow mould (Table 1). Against a growth of 84.73 mm in the control the radial growth of the fungus in dual cultures with these three isolates ranged from 34.79 mm to 43.59 mm. Thus, the growth of the pathogen was reduced by over 50 per cent. However,

Table 1 Biocontrol potential of selected isolates of fluorescent pseudomonads in *in vitro.*

S.No	Bacterial isolates	Pathogenic fungi															
		Sepedonium sp.		*l-'uxtirium* sp.		*Trichoderma* sp.		*Neurospora* sp.		I*'erticitliitm* sp.		*Diehliomyces* sp.		*Mycogone* sp.		C*'ladobotryum* sp.	
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1	CI_L.	46.67	34.79	90.00	00.00	90.00	00.00	82.17	00.00	53.23	30.69	75.43	00.00	81.97	00.00	90.00	00.00
2	$Cl!_b$	43.57	42.10	45.03	38.70	44.60	45.42	40.77	39.73	51.20	31.10	42.77	34.67	35.60	44.87	90.00	00.00
3	$Clll_b$	88.33	00.00	84.03	00.00	90.00	00.00	82.33	00.00	79.77	00.00	75.23	00.00	82,77	00.00	90.00	00.00
4	CIV,	83.00	00.00	82.30	00.00	90.00	00.00	81.37	00.00	81.17	00.00	74.70	00.00	82.87	00.00	90.00	00.00
5	CV_a	83.67	00.00	40.63	44.67	90.00	00.00	46.20	39.50	39.70	44.67	75.83	00.00	81.70	00.00	90.00	00.00
6	$ClII_d$	85.03	00.00	80.70	00.00	90.00	00.00	81.87	00.00	81.73	00.00	75.90	00.00	82.59	00.00	90.00	00.00
7	CVl_a	42.10	43.59	31.79	54.83	90.00	00.00	80.67	00.00	81.87	00.00	75.59	00.00	55.27	25.23	90.00	00.00
8	Check	84.73	00.00	80.87	00.00	90.00	00.00	80.80	00.00	83.87	00.00	76.29	00.00	81.83	00.00	90.00	00.00

CD at 5%			
	For Isolates	(a)	0.85
	For pathogenic fungi	(b)	0.85
	For radial growth and inhibition zone	(c)	0.43
		(axb)	2.41
		(bxc)	1.20
		(axe)	1 .20
		(axbxc)	3.40

1. Diameter of mycelial growth (mm)
2. Diameter of inhibition zone (mm)

Table 2 Biocontrol potential of selected isolates of fluorescent pseudomonad.s against pathogens in mushroom beds.

Isolates	Treatments	Yield in kg/qtl compost (30 days harvest)					
		Sepedonium sp.	*Fusarium* sp.	*Neurospora* sp.	*VerliciUium* sp.	*Mycogone* sp.	*Trichoderma* sp.
CIc	SI - 100 mix 3 spray	6.70			9.08		
	S2 - 200 ml x 3 spray	6.02			4.62	-	-
	Ml -300ml mixed	11.08	-	-	7.94		
	M2 - 600 ml mixed	6.26			4.08		
	CP - Control (pathogen only)	4.60	-	-	5.98	-	-
	CA - Control {*A. bisporus* only)	14.23			14.23		
CII$_b$	SI - 100ml x 3 spray	11.30	11.10	11.42	12.94	9.46	11.62
	S2 - 200 ml x 3 spray	9.16	9.70	10.60	10.44	6.62	4.40
	Ml -300 ml mixed	11.42	10.18	12.58	11.56	8.48	10.22
	M2 - 600 ml mixed	9.92	4.94	9.56	9.24	5.42	3.40
	CP - Control (pathogen only)	4.60	5.92	7.56	5.98	2.34	3.94
	CA - Control (*A. bisporus* only)	14.23	14.23	14.23	14.23	14.23	14.23

cont'd..

Table 2 contd...

Isolates	Treatments	Yield in kg/qtl compost (30 days harvest)					
		Sepedonium sp.	*Fusarium* sp.	*Neurospora* sp.	*Verticillium* sp.	*Mycogone* sp.	*Trichoderma* sp.
CV_a	SI - 100 mix 3 spray		13.12	16.78	8.98		
	S2 - 200 ml x 3 spray	-	12.48	6.74	5.76	-	-
	Ml - 300 ml mixed	-	8.18	11.44	9.92	-	
	M2 - 600 ml mixed	-	3.74	9.10	7.38	-	-
	CP - Control (pathogen only)	-	5.92	7.56	5.98	-	-
	CA - Control (*A. bisporus* only)	-	14.23	14.23	14.23	-	-
CVI_a	SI - 100 mix 3 spray	8.66	9.36		-	12.02	-
	S2 - 200 ml x 3 spray	6.58	6.80	-	-	5.40	-
	Ml - 300 ml mixed	9.80	8.88	-	-	8.60	-
	M2 - 600 ml mixed	6.86	5.00	-	-	5.08	-
	CP - Control (pathogen only)	4.60	5.92	-	-	2.34	-
	CA - Control (*A. bisporus* only)	14.23	14.23	-	-	14.23	
	CD at 5%	1.25	1.40	0.90	0.75	1.50	2.15

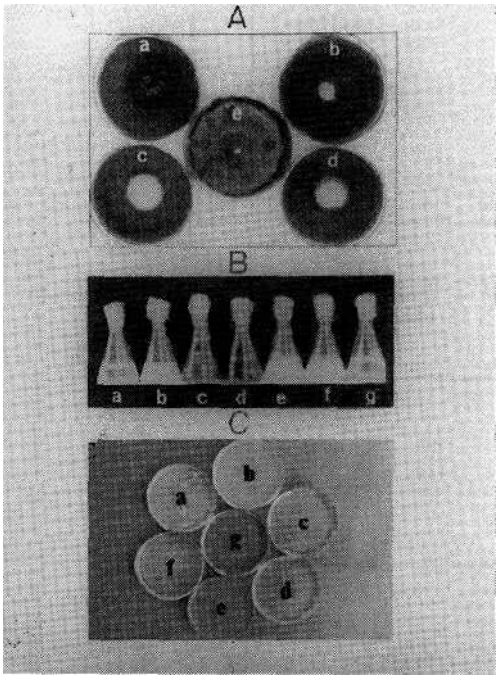

Fig. 1. Production of siderophores by pseudomonads

(A) Siderophores production in chrome Azurols Assay
Isolates (a) CIc (b) Cllb (c) CHIb (d) CIVa (e) CVa
(B) Siderophores production in SSGM broth
Isolates
(a) CHId No siderophores production
(b) CIc
(c) Cllb
(d) CHIb Siderophore production
(e) CIVa
(f)CVa
(g) Uninoculated (Control) No change in color
(C) Effect of iron on siderophores produced by isolates of fluorescent pseudomonads
Isolates
(a) CHId No change in pigment (Color)
(b) CIc
(e) Cllb
(d) CHIb Pigment (Color) changed light to dark red
(e) CIVa
(f)Cva
(g) Uninoculated (Control) Natural pigment, No change in color.

isolates CHIb, CIVa CVa and a non-siderophore producer isolate CHId were totally ineffective. Against *Fusarium* sp., isolate Cllb, Cva and CVIa were effective. These isolates reduced growth of *Fusarium* by about 50 to 60 per cent. *Trichoderma* sp., causing green mould and itself a strong antagonist against soil borne pathogens of plants (Weller & Cook, 1986) was effectively reduced by fluorescent pseudomonads isolate Cllb. The remaining isolates were ineffective and run over by *Trichoderma* sp. itself. The *Neurospora* sp., which causes the fire mould was significantly controlled by only two isolates i.e, Cllb and CVa and the rest were ineffective. *Verticillium* sp., causing dry bubble disease, was reduced by isolate CIc Cllb and

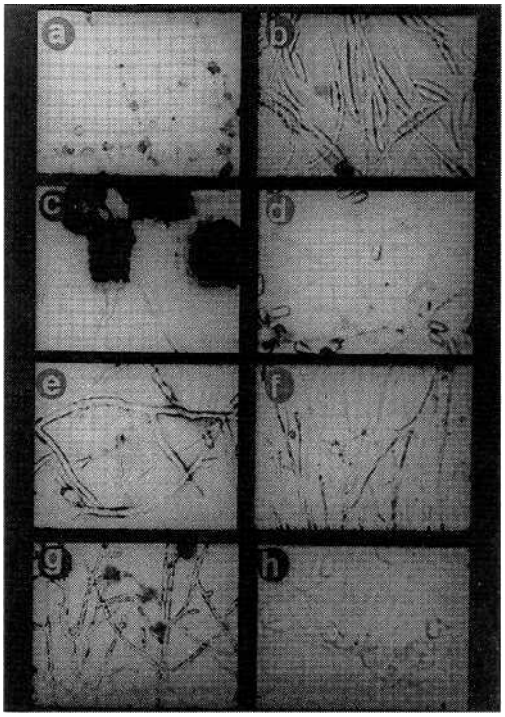

Fig. 2. Microphotographs of pathogenic fungi

(A) *Sepedoniwn chrysosporium*
(a) Hyphe (b) Aleuroconidium
(B) *Fusarium moniliforme*
(a) Hypbae (b) Macroconidia (c) Microconidea
(C) *Trichoderma harzianum*
(a) Hyphae (b) Phialospores (c) Phialids
(D) *Neurospora crassa*
(a) Hyphae (b) Conidium
(E) *Verticilliumfungicola*
(a) Hyphae (b) Conidiophores (c) Conidia
(F) *Diehliomyces*
(a) Hyphae (b) Chlamydospores
(G) *Mycogone perniciosa*
(a) Hyphae (b) Conidiophores (c) Chlamydospores
(H) *Cladobotryum dendroides*
(a) Hyphae (b) Conidiophores (c) Conidia

CVa. Each reduced growth of the fungus significantly without significant differences within themselves.

Diehliomyces sp., known to cause false truffle, was reduced only by isolate Cllb. However, *Mycogone perniciosa* causing wet bubble disease was inhibited by isolate Cllb and CVIa. Isolate Cllb was significantly superior to isolate CVIa.

The test pathogen *Cladobotryum dendroides* which causes cob web was not checked by any of the seven isolates tested.

The results thus have clearly revealed that among the microflora existing in the casing those having antagonistic potential could be used for management of different fungal diseases of *A. bisporus.* The earlier studies by Baker (1987), Jhune *et al.* (1 990), Kloepper (1992) and Ablawat & Rai (1997) support the usefulness of bacterial flora in control of pathogens affecting yield of *A. bisporus.*

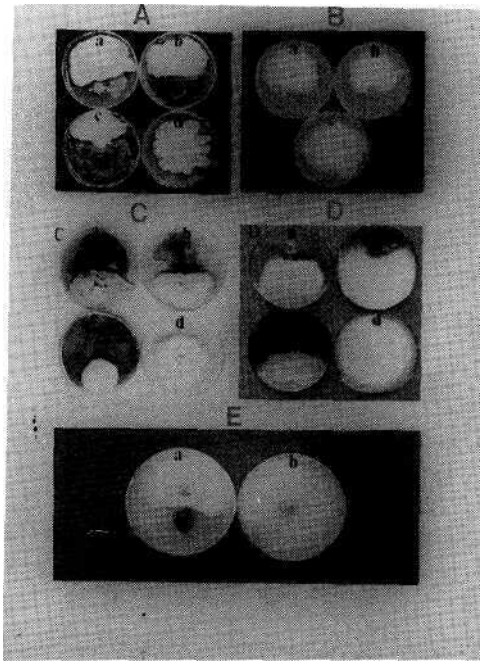

Fig. 3. Biocontrol potential of pseudomonads isolates against fungi parasitizing^, *bisporus*

(A) Antibiosis *ofSepedonium chrysosporium* with bacterial isolates
(a)CIc (b)CIIb (c)CVIa (d) Control

(B) Antibiosis *ofFusarium moniliforme* with bacterial isolaties
(a) Cllb (b) CVa (e) CVIa (d) Control

(C) Antibiosis *ofNeurospora crassa* with bacterial isolates
(a) Cllb (b)CVa (c) Control

(D) Antibiosis of *Verticillium fungicola* with bacterial isolates
(a)CIc (b)CIIb (e)CVa (d) Control

(E) Antibiosis of *Trichoderma harziamim* with bacterial isolaties
(a) Cllb (b) Control

In order to outline the potential of bacterial isolates in control of pathogens during the mushroom cultivation cycle, the isolates found promising against specific pathogens in *in vitro* studies were further tested in casing as detailed under Materials and Methods. The yield obtained from the treatments and control are presented in Table 2.

Of the three isolaties CIc Cllb and CVIa, the isolates Cla and Cllb gave significantly higher yield with treatments MI in control of pathogens *Sepedonium.* However, the yield obtained from the isolate Cllb With treatments Ml and SI were statistically at par. The different treatments of isolate CVIa enhanced the yield significantly as compared to control (CP) where pathogen was inoculated at casing. The significantly higher yields were obtained adding isolate Cllb (SI, SII and MI), CVa (SI and SII) and CVIa(SI) in the mushroom beds inoculated with *Fusarium* sp. at casing.

The fungus *Neurospora* was best controlled with the use of isolates Cllb (SI and MI) and CVa (SI and MI). In case of *Verticillium* sp., the isolate Cllb with the treatments SI, SII and MI was found most effective in terms of yields obtained, however, all the treatments of Cllb and

CVI, were significantly superior over the control (pathogen only) but the treatments with SI of CVIa was found to be most effective. The maximum yield was recorded using isolate CVIa with treatment SI followed by Cllb SI against *Mycogone* sp. *Trichoderma* sp., was also controlled effectively by using isolate Cllb (SI and MI).

In general the bacterial isolate Cllb with the three sprays of bacterial suspension of 100 ml each and 300 ml bacterial suspension mixed at casing were found most promising in terms of enhanced mushroom yield over control of pathogens (CP), *Sepedonium, Fusarium, Neurospora, Verticillium, Trichoderma* and *Mycogone.*

ACKNOWLEDGEMENTS

The first author is grateful to the Directorate of Experiment Station, GBPUAT, Pantnagar for providing fellowship and necessary facilities to carry out the present work.

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