BIOLOGICAL CONTROL OF RICE BLAST DISEASE WITH ANTAGONISTIC BACTERIA UNDER EGYPTIAN CONDITIONS

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Abstract

Forty two bacterial isolates were isolated from rice leaves of different Egyptian rice cultivars and some other plants in 1995. Morphological characters of both colony and cell shape, reaction of Gram Staining and KOH test as preliminary identification tests showed that, most of the isolates belong to *Bacillus* spp. and *Pseudomonas* spp. More confirmative identification tests are needed.

Twenty isolates showed inhibitory effect against *Pyricularia oryzae* Cav.,the causal organism of blast disease in both laboratory and greenhouse tests, sixteen isolates showed the highest mycelial reduction ranging from 53.6 to 40.5%. In greenhouse tests,all isolates were effective comparing with the check; however, 14 of them were effective for minimizing blast disease infection under all application methods (seed treatment, spray 3 DBI and 3 DAI).Seven isolates were more effective when used as seed treatment or sprayed 3 DBI. On the other hand,three other isolates were effective as seed treatment and spray 3 DAI.

Regardless of the insufficient effect under laboratory test, some isolates showed satisfactory effect under greenhouse test.

INTRODUCTION

Rice is one of the most important food crops all over the world. Blast disease caused by the fungus *Pyricularia oryzae* Cav. is considered the most serious production constraint in Egypt. Because of the ability of the fungus to evolve new races, control of blast through breeding for resistance has had only partial success in favorable conditions.

Therefore, it is important to find additional and/or alternative disease management strategies that are nonpolluting and inexpensive. The use of microorganisms and their metabolic products as biological control agents has been considered for a long time (Mew, 1990; Gnana *et al.*, 1992;). Recently, greater efforts are directed towards research and development of biological control techniques. In case of foliar diseases, Leben (1985) suggested that it is best to look for and select antagonists with "special affinity" for the leaf or one that would multiply on the leaf proper. These antagonists showed considerable success as control agents against foliar pa-

thogens (Sleesman and Leben, 1976; Leben *et al.*, 1965; Andres, 1992; Leben, 1985). From these reports it is likely that blast lesions harbor epiphytic population comprising possible antagonists.

Examining microorganisms for their suppressive properties of *P.oryzae* growth *in vitro* indicated that many species were promising candidates for use as biological control agents (BCA) for rice blast disease (Sy *et al.*, 1983).

Early work at the International Rice Research Institute (IRRI) revealed that there was a diverse microbial population showing antagonistic activities to most rice fungal pathogens (Mew and Rosales, 1986). Studies conducted by Rosales *et al.* (1993) revealed that members of at least four genera (*Bacillus, Pseudomonas, Serratia* and *Erwinia*) have antagonistic activity against some rice fungal pathogens. Three strains identified as *B.subtilis* inhibited the mycelial growth of eight fungal pathogens including *P.oryzae, H.oryzae* and *F.moniliforme*. Seed bacterization with these bacterial strains provided a sheath blight protection of 4.5 to 73% in the glasshouse trial.

No available information on the association of antagonistic bacteria with blast lesions as well as their effect on blast disease development in Egypt. Accordingly, this work was conducted at Rice Research & Training Center, Plant Pathology Department to search for and to test the efficiency of some bacterial isolates as biocontrol agents, against *P.oryzae*, the causal of rice blast disease.

MATERIALS AND METHODS

1. Isolation of bacteria associated with leaf blast lesions

Leaves of different rice varieties were collected from different locations in the Nile delta, Egypt in the 1995 season. Lesions of each leaf sample were cut and homogenized. Serial dilution up to 10^{-6} was prepared, in which 0.1 ml of each dilution was plated unto 4 replicates of plain agar following the standard dilution plating technique. Different colony types of bacteria were transferred to PDA slants as pure cultures for further studies and uses.

2.Characterization and identification of the isolates under testing

Gram stain reaction and cell morphology were observed on colony grown on nutrient agar slants for 24 h at 28^{o}C. The Gram reaction was determined following the procedures given by Schaad (1980). Cell dimensions were measured using the

ocular micrometer attached to the eyepiece of the microscope.

3. *In vitro* test of bacterial isolates:

Bacteria isolated from different rice leaf samples were screened for their antagonistic activity against *Pyricularia oryzae* in the laboratory using the dual agar culture test (Mew and Rosales, 1986).

Isolates were individually tested for their ability to inhibit mycelial growth of the pathogen, in quadruplicates at 28°C.

Antagonism between mycelial growth of *P.oryzae* and each bacterial isolate was assessed after seven days of incubation by measuring the mycelial growth diameter of the pathogen towards the line of potential bacterial antagonists. Plates with the fungus alone served as the control.

Percentage reduction of mycelial growth (M.G.) for each isolate was obtained according to the following formula:

$$\% \text{ of MG. reduction} = \frac{\text{MG. of check- MG. of treatment}}{\text{M.G. of check}} \times 100$$

4. Greenhouse test of selected bacterial antagonists

A virulent isolate of *P.oryzae* from the culture collection kept at the Plant Pathology Department, RRTC, was transferred into banana medium slants for sporulation. Fungal inoculum was prepared by adding 10 ml of sterile water per tube of the pure culture of *P.oryzae*, and shaken to free the spores.

Preparation of bacterial suspension

Bacterial isolates were growth on PDA plates for 24 hours at 28°C. Bacterial suspension was prepared by adding 20 ml of sterile distilled water per plate and scraping the growth with a wire loop. The concentration of bacterial suspension was adjusted to 10^9 colony forming units (CFU) per ml.

Seed treatment

Seeds of rice, Giza 176 cv. were surface sterilized by immersion in 2.5% calcium hypochlorite solution for three minutes and then rinsed in three changes of sterile distilled water. The seeds were soaked for 24 hours into the previously pre-

pared bacterial suspension and sown in 9 cm diameter plastic pots (10 seeds/pot).

Seed treatment:

Seedlings at 3-4 leaf stage (14 days old) were sprayed with a 10^5 spores ml^{-1} suspension of *P.oryzae*, under small cages. There were four main treatments as follows:

1. Seed bacterization with individual bacterial isolates.
2. Spray with bacterial isolates 3 days before the challenge inoculation with the fungus (3 DBI).
3. Spray with bacterial isolates 3 days after the fungal application (3 DAI).
4. Rice seedling sprayed with spore suspension of *P.oryzae* alone, as a check.

In all treatments disease infection was measured 7 days after inoculation, by counting number of type 4 lesions/25 rice leaves.

RESULTS AND DISCUSSION

Isolation and identification of bacterial isolates:

A total of forty two bacterial isolates were collected, thirty two isolates were obtained from different Egyptian rice cultivars, while ten isolates were isolated from different plant extracts.

The isolates were classified depending on their phenotypic features. Twenty five isolates were Gram-positive, while only three appeared as Gram-negative. The rest of isolates indicated no clear cut differentiation. Eight isolates were spherical (0.5-1.0 Um in diam), while 17 isolates were rod-shape. Six isolates of them ranged from 1to 2 Um and eleven isolates were 2-3 Um in length. On the other hand, there were six isolates punctiform (Table 1). Based on these morphological features and reaction to staining, most of the bacterial isolates corresponded to the genus *Bacillus* in Bergey's Manual (Sneath, 1984). However, further and more definitive tests are needed to confirm this prelimemary identification.

Antagonism of the bacterial cultures *in vitro*:

Thirty two isolates were selected to perform both laboratory and greenhouse tests. Twenty nine isolates showed antagonistic activity towards *Pyricularia*

oryzae; sixteen of them resulted in a reduction of mycelial growth ranging between 40.5 and 53.6% with no significant difference (Tables 2, 3 & 4). The remaining thirteen isolates, caused the lowest mycelial growth reduction ranging between 3.3 and 22.8%. This indicates that antibiosis was involved in inhibiting the growth of the pathogen. This form of biological control could work in two mechanisms : 1) reduction or prevention of germination, 2) slowing down of growth due to starvation, antibiotics or bacteriocin according to Homma and Suzui (1989).

Table 1. Some morphological characters, staining reaction and cell length of the bacterial isolates.

No.	Rod Shape				Spherical		Punctiform	
	1-2 Um		2-3 Um		0.5-10 Um	Um		
	I. Gram positive :							
1	9	B*	3	A*	1	A*	10	C*
2	13	A	8	A	11	A	21	A
3	14	A	12	A	19	C	25	A
4	28	A	15	A	24	D	29	A
5	33	A	16	A	30	B	32	A
6	34	A	18	A	38	A	40	A
7			22	A	39	A		
8			23	A	41	D		
9			26	A				
10			36	A				
11			42	A				
	II- Gram negative : 7,17,27 III - Koh test : All bacterial isolates were negative except Nos. (1,11,14, 15, 19, 24, 27 and 41)							

*** Morphological character :**

A = Creamy-flat-membranous
B = Yellow-flat-erose
C = Red-abundant growth
D = Yellow-black pigmentation

Effect of seed bacterization and spray application on blast development:

In greenhouse tests blast disease protection afforded by either seed bacterization and spray application three days before or after inoculation with spore suspension of *P.oryzae* is presented in tables (2, 3 & 4). The results showed that, all bacterial strains were effective for minimizing the diseae infection under the application methods.

Out of twenty nine isolates used for seed treatment in seedling test, twenty five isolates were very effective in controlling blast disease. The protective efficiency ranged from 98.5 to 70.0%. All these isolates gave significant reduction comparing with the check treatment, whereas the remaining isolates afforded 53% protective efficiency over the check (Tables 2, 3 & 4). The bacterial strain RRC-3 showed the most prorective ability where only one lesion developed on 100 leaves under this method of application.

The seedlings sprayed individually with bacterial isolates three days after inoculation with *P.oryzae* showed positive results. Twenty two isolates afforded reduction of infection severity ranging from 97.8 to 70.0%. Isolates (RRC-9, 14, 16, 17, 18, 32, 33 and 38) resulted in maximum control of blast disease severity (1-3 lesions/100 leaves) without significant difference. On the other hand, the seedlings sprayed with bacteria three days before inoculation, with the blast fungus, showed lower efficiency for all isolates in disease reduction than both seed treatment and spray 3 DAI methods as the protective efficiency ranged from 78.0 to 60.0% for ten isolates (Tables 2, 3 & 4).

This study demonstrated that, bacterial treatments with selected strains protected rice plants from blast disease under greenhouse. This finding is in agreement with those of Gnanamanickam and Mew (1992), and Homma *et al.* (1989), who mentioned that antibiotics produced by *Pseudomonas cepecia*, showed high activity against several species of fungal pathogens including *Pyricularia oryzae, Rhizoctonia solani* and *Verticillium dahliae.*

It is too early to state that biological control could be used as one of the disease management options, however, it is worthwhile to explore the possibility of using these antagonistic bacteria in managing rice blast disease.

However, there is a possibility that such an antagonistic effect occurs in nature, and more research is needed to idenitfy the role of epiphytes in controlling rice blast.

Table 2. Effect of bacterial isolates *in vitro* and *in vivo* against *Pyricularia oryzae* .

No.	Isolate No.	% of M.R. in Lab.*	Severity of blast infection in greenhouse		
			S.T. **	S 3 DBI @	S. 3 DAI #
	A. Effective bacterial isolates in all application methods:				
1	RRTC 3	52.5	1.0	17.3	12.0
2	RRTC 7	42.4	15	26.0	8.3
3	RRTC 8	47.1	8.3	18.3	6.3
4	RRTC 9	53.6	7.8	21.0	1.5
5	RRTC 13	48.4	11.3	53.0	9.0
6	RRTC 16	50.4	15.0	47.0	2.5
7	RRTC 17	48.3	6.8	29.0	1.8
8	RRTC 18	52.8	3.5	34.0	1.5
	B. Effective bacterial isolates in greenhouse only:				
9	RRTC 11	1.4	15.8	23.3	13.0
10	RRTC 20	6.1	13.5	43.0	8.1
11	RRTC 21	16.2	9.8	19.0	3.8
12	RRTC 27	4.1	24.8	27.8	13.8
13	RRTC 28	1.4	6.5	50.5	14.0
14	RRTC 32	1.5	14.3	20.3	2.0
15	RRTC 38		19.3	31.3	2.5
16	RRTC 14	3.3	11.3	53.3	25.3
17	RRTC Con-	0	66.8	66.8	66.8
	trol	10.3	8.3	22.7	8.5

* M.R. = Mycelial reduction
** ST = Seed treatment
@ S 3 DBI = Spray three days before inoculation
# S 3 DAI = Spray three days after inoculation

Table 3. Effect of bacterial isolates *in vitro* and *in vivo* against *Pyricularia oryza* .

No.	Isolate No.	% of M.R. in Lab.*	Severity of blast infection in greenhouse S.T. **	S 3 DBI @	S. 3 DAI #
	A. Effective bacterial isolates in all application methods:				
1	PRTC 22	49.2	17.0	31.3	35.3
2	PRTC 23	50.9	12.3	36.3	48.0
3	PRTC 34	52.0	15.0	33.3	24.3
	B. Effective bacterial isolates in greenhouse only:				
4	PRTC 19	3.3	11.3	53.3	25.3
5	PRTC 25	9.4	14.5	14.3	20.3
6	PRTC 29	5.5	7.5	50.0	24.0
7	PRTC 37	9.4	4.5	41.3	70.0
	Control	0	66.8	66.8	66.8
	LSD 5%	10.3	8.3	22.7	8.5

Table 4. Effect of bacterial isolates *in vitro* and *in vivo* against *Pyricularia oryza*.

No.	Isolate No.	% of M.R. in Lab.*	Severity of blast infection in greenhouse S.T. **	S 3 DBI @	S. 3 DAI #
	A. Effective bacterial isolates in all application methods:				
1	PRTC 12	47.1	8.5	68.5	11.3
2	PRTC 14	53.2	9.0	89.3	3.3
3	PRTC 15	44.9	6.8	75.0	13.3
	Control	0	66.8	66.8	66.8
	B. Effective bacterial isolates with spray either 3 DBI or 3 DAI in greenhouse and in lab.				
1	PRTC 33	40.5	31.3	26.3	3.3
2	PRTC 38	53.5	18.0	42.8	12.5
3	PRTC 24	12.7	26.3	25.0	9.3
4	PRTC 38	1.5	19.3	31.3	2.3
	Control	0	66.8	66.8	66.8
	LSD 5%	10.3	8.3	22.7	8.5

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المقاومة الحيوية لمرض اللفحة فى الأرز بإستخدام البكتيريا تحت الظروف المصرية

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تم عزل وتنقية إثنين وأربعين عزلة بكتيرية من أوراق الأرز ونباتات اخرى – موسم ١٩٩٥ من أصناف مختلفة. أجريت على تلك العزلات الاختبارات الأولية لتعريف :

أ. الشكل الخارجى لكل من نمو المزارع، وشكل الخلايا البكتيرية.

ب – اختبارات صبغ الخلايا البكتيريه بصبغة جرام.

جـ . إختبار هيدروكسيد البوتاسيوم.

وقد أظهرت هذه الاختبارات أن معظم العزلات تتبع إما جنس *Bacillus spp.* أو جنس *Pseudomonos* لكن يتطلب اكتمال التعريف إجراء اختبارات حيوية أخرى .

نتائج إختبارات المعمل:

نتيجة تلك العزلات البكتيرية ضد الفطر المسبب لمرض اللفحة Pyricularia فى المعمل أوضحت أن هناك عشرين عزلة أعطت نتائج مبشرة فى إيقاف نمو الفطر، منها ستة عشر عزله أعطت أعلى نسبة تضاد وإيقاف النمو الميسليومى للفطر على البيئة بنسبة من ٤٠,٥ إلى ٥٣,٦٪.

نتائج إختبارات الصوبة:

أظهرت جميع العزلات البكتيرية فعالية بدرجات متفاوتة ضد تكشف وظهور المرض على بادرات الأرز (المعداه بجراثيم الفطر).

حيث أعطت أربعة عشر عزلة فعالية كبيرة فى إيقاف ظهور المرض تحت جميع الطرق التطبيقية (معاملة بذرة – الرش بالمحلول البكتيرى مثل العدوى بالفطر بثلاثة أيام – أو المعاملة بالبكتيريا بعد العدوى بالفطر بثلاثة أيام). بينما أظهرت سبعة عزلات فعالية إما بطريقة معاملة البذرة أو بالرش قبل العدوى بالفطر لكن ثلاث عزلات أخرى كانت فعالة من خلال إما معاملة البذرة أو الرش بعد العدوى بالفطر.

وعلى الرغم من ضعف تأثير بعض العزلات البكتيرية فى إختبارات المعمل إلا أنها أظهرت فعالية ملحوظة فى إيقاف المرض عند إختبارها على النباتات فى الصوبه.