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Effects of antibiotics on biological control agents and their efficacy to control rice sheath blight (*R. solani* AG-I.1)

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Effects of antibiotics on the growth of four strains of biological control agents (BCA) B-916 (*Bacillus subtilis*), P7-14 (*Pseudomonas fluorescens),* P9409 (*P. resinovorans)* and P10353 *(P. malculicola)* were tested by growing them on peptone potassium nitrate medium (PPM) ranged from 0.5 to 1000 ug/mL of four antibiotitics: ampicilin, hygromycin, kanamycin and rifampicin. Antibiotics affected the growth of some BCAs but not others. Some BCAs were able to withstand high concentrations of antibiotics and thus their growth was not affected. Sheath blight of rice (*Oryza sativa*) caused by *Rhizoctonia solani* AG-I.1 was effectively controlled by the antibiotics, BCAs and mutants depending on the compatibility of the BCA and antibiotics. The three antibiotics at 50 ug/mL) reduced sheath blight by 60-80% as measured by area under the disease progress curve (AUDPC). B-916 and P9409 controlled the disease over 75%, whereas P7-14 and P10353 were less effective. B-916 + ampicilin, P10353 + rifampicin and P9409 + rifampicin. (25 ug/mL) was mixed with a BCA (0.5 X 10^7 CFU/mL) were sensitive resulted, disease suppression was consistently weakened.When an antibiotic and a tolerant BCA were combined as P7-14 + hygromicin and P9409 + hygromycin, their efficacy was unchanged or enhanced. Compared with the parent BCA, bio-control efficacy of hyg^R-714 and hyg^R-9409 was significantly enhanced (P<0.05) or unchanged in other cases.

Key words: Antibiotics, Sheath blight, Biological Control Agents.

Introduction

Control of rice diseases biologically with antagonistic microbial organisms has been studied extensively and is considered a promising technology (Mew *et al.* 2003). Many factors have been shown to affect the efficacy of bio-control agents (BCAs), but there have been few studies on effects of pesticides disease bio-control (Zhao *et al.* 2002). Some antibiotics have been reported to be effective against bacterial diseases of crops. It is

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important to determine whether antibiotic applications in the field affect disease suppression by BCAs. In bio-control studies, mutation with antibiotics has been exploited to obtain bacterial mutants with acquired or increased antagonism to pathogens (Georgakopoulos *et al.* 1994). Little information has been on bacterial BCA mutations in relation to bio-control of diseases in rice systems and no work has been done on this in Nigeria. The objectives of this study were to (a) analyze the direct effects of different antibiotics on the growth of BCAs, (b) obtain BCA mutants with enhanced bio-control activity and (c) examine the effects of antibiotics on rice sheath blight and BCA function.

Materials and methods

The effect of antibiotics; ampicilin, hygromycin, kanamycin and rifampicin on the growth of different bacterial BCAs of four strains, B-916 (*Bacillus subtilis*), P7-14 (*Pseudomonas fluorescens),* P9409 (*Pseudomona resinovorans) and P10353 (Pseudomona malculicola)* were tested on peptone potassium nitrate medium (PPM) at different concentrations ranged from 0.5 to 1000 ug/mL. The mutants of amp^R –B916, hyg^R-714, hyg^R-9409, rif^R-10353 and rif^R-9409 were also tested their growth on mentioned antibiotics. The experiment was conducted by using two factor factorial experiments in Completely Randomized Design (CRD) with six replications. The colony growth of each strain was measured and computed statistically analysis, treatment means were compared using Duncan's multiple range Test (DMRT) at P>0.05. The efficacy of selected BCAs of B-916, Amp^R-B916, Amp.50, B-916 + A25, Hyg^R-714 + H25, Hyg^R-714 + H25 was tested to control sheath blight of rice (*Oryza sativa*) caused by *Rhizoctonia solani* AG-I.1 compared to the non-treated check. Sheath blight severity was recored as lesion area in mm^2 and compared the difference between BCA antibiotic treatments. The experiment was conducted by using Randomized Completely Block Design (RCBD) with nine replications. The lesion area was measured and computed statistically analysis, treatment means were compared using Duncan's multiple range Test (DMRT) at P>0.05. Area under Disease Progress Curve (AUDPC) values were calculated from the means in each treatment.

Results and discussions

The four antibiotics affected growth of the four BCAs quite differently (Table 1). Kanamycin had a profound effect on B-916, P7-14 and P9409, which could not grow at 3 ug/mL, but it did not affect the growth of P10353 at concentrations of more than 250 ug/mL. Rifampicin at the highest concentration used in the experiment (1000 ug/mL) did not influence the growth of P7-14, but the other three bacteria stopped growing at 3ug/ mL (Tan and Mew, 2001). However, apart from that of B-916, the growth of the other three bacteria was not affected by ampicilin at rather high concentrations (>100ug/mL). Some BCAs mutated easily, such as P7-14 and P9409 on hygromycin, P10353 and P9409 on rifampicin and B-916 on ampicilin (Table 1). Mutants of these BCAs resistant to 1000ug/mL of the antibiotics were obtained. In some situations some BCAs such as B-916 and P9409 on kanamycin and B-916 on rifampcin Pathogenicity, antagonicity and cell growth of the mutants were not significantly changed compared with parent bacteria, but the growth of amp^R -916 decreased markedly and antagonisticity of hyg^R -714 and hyg^R-714 and hyg^R-9409 increased markedly (P<0.05).

The three antibiotics (50 ug/mL) reduced sheath blight by 60-80% as measured by area under the disease progress curve (AUDPC). B-916 and P9409 [10^7 colony-forming units (CFU) mL^{-1}] controlled the disease by more than 75%, whereas P7-14 and P10353 were less effective. When an antibiotic (25 ug/mL) was mixed with a BCA (0.5 X 10^7 CFU/mL) sensitive to it, disease suppression was consistently weakened, for example, B-916 + ampicilin, P10353 + rifampicin and P9409 + rifampicin. When an antibiotic and a tolerant BCA were combined, their efficacy was unchanged or enhanced, as shown by P7-14 + hygromicin and P9409 + hygromycin. Compared with the parent BCA, bio-control efficacy of hyg^R -714 and hyg^R -9409 was significantly enhanced (P<0.05) or unchanged in other cases (P>0.05). The combined spray of mutant with its inducer antibiotic markedly improved bio-control efficiency in some cases, but it was not altered in other cases based on the control effect of the mutant (Mew *et al*. 1993).

Table 1. Effect of antibiotics on growth of BCAs and mutants

Antbiotc	**Highest growth** [a]			**Mutant growth on** [b]			
	BCA	concentration (ug/mL)		Mutant	PPM	PPM + antibio	Wild type on PPM
		BCA	Mutant				
Ampicilin	B-916	1	1000	amp^R–B916	341b	243c	735a
	P7-14	500	-				
	P10353	500	-				
	P9409	250	-				
Hygromycin	B-916	10	100				
	P7-14	50	1000	hyg^R-714	752a	755a	767a
	P10353	250	-				
	P9409	50	1000	hyg^R-9409	596a	582a	581a
Kanamycin	B-916	1	10				
	P7-14	1	250				
	P10353	500	-				

	P9409	1	10				
Rifampicin	B-916	0.5	3				
	P7-14	500	-				
	P10353	10	1000	rif^{R}-10353	654a	633a	630a
	P9409	1	1000	rif^{R}-9409	611a	573a	581a

[a]BCA growth was considered not affected and mutation was not performed when it could grow at 250 mg/Ml. [b] Each value is the mean of six replicates. Means (number of colonies per plate) in a row with the letter are not significantly different (P>0.05) by Duncan Multiple Range Test (DMRT).

Table 2. Comparison of sheath blight severity (lesion area in mm^2) difference between BCA antibiotic treatments[a]

Treatment	**Days after treatment**					
	3	4	5	6	7	AUDPC
B-916	5.4c	12.3c	31.2c	98.7c	163.3c	226.6
Amp^{R}-B916	1.0c	8.0c	24.7cd	72.7c	124.0c	167.9
Amp.50	4.3c	12.3b	29.7c	73.7c	115.3c	175.5
B-916 + A25	15.3b	33.4b	86.7ab	172.0b	248.5b	423.8
AMP^{R}-B916 + A25	3.0c	7.7c	19.0d	40.0d	75.3d	105.9
Check	22.0a	72.7a	145.0a	262.3a	363.3a	672.7
7-14	16.3a	45.7b	99.0ab	124.3bc	201.7b	363.3
Hyg^{R}-714	14.7ab	33.7b	55.3c	96.7c	146.5c	266.3
Hyg.50	5.3b	15.7c	45.7c	86.7c	127.7c	214.6
7-14 + H25	22.0a	58.3ab	75.3bc	135.0b	191.3b	375.3
Hyg^{R}-714 + H25	8.0b	21.7c	53.0c	89.3c	130.3c	227.2
Check	22.0a	72.7a	145.0a	262.3a	363.3a	672.7
10353	9.7b	34.0b	62.7b	104.3b	191.0c	301.4
Rif^{R}-10353	10.3B	22.7c	53.0b	94.3b	150.0c	250.2
Rif. 50	5.0b	17.3c	55.3b	89.7b	158.3c	244.0
10353 + R25	8.0b	30.7b	74.7b	124.3b	253.3b	360.4
Rif^{R}-10353 + R25	7.7b	18.5c	35.3c	61.4c	98.7c	168.4
Check	22.0a	72.7a	145.0a	262.3a	363.3a	672.7
9409	6.5c	15.7c	35.3c	84.7c	173.5c	225.7
Rif. 50	5.0c	17.3c	55.3bc	89.7c	158.3c	243.9
9409 + R25	11.7b	31.3b	66.0c	156.3b	251.0b	389.9
Rif^{R} -9409 + R25	1.3c	22.4c	51.3bc	93.3c	135.7c	240.5
Check	22.0a	72.7a	145.0a	262.3a	363.3a	672.7
9409	6.5a	15.7b	35.3c	84.7b	173.5b	225.7
Hyg^{R}-9409	0c	5.3c	10.7d	39.7c	90.3c	100.9
0. Hyg. 50	5.3b	15.7b	45.7b	86.7b	127.7c	214.6
9409 + H25	1.7b	8.3c	23.7d	35.3c	68.7d	102.5
Hyg^{R} -9409 + H25	1.3b	6.4c	21.3d	33.3c	45.7d	89.5
Check	22.0a	72.7a	145.0a	262.3a	363.3a	672.7

[a] Each value is the mean of nine replicates. Means in a column with the same letter are not significantly different (P>0.05) by Duncan Multiple Range Test (DMRT). Area under Disease Progress Curve (AUDPC) values were calculated from the means in each treatment.

Conclusion

Antibiotics affected the growth of some BCAs but not other. Some BCAs tolerated high concentrations of antibiotics and thus their growth was not affected. Of the five mutants obtained from the BCAs, two had enhanced antagonisticity and one had lowered growth capability. No mutant developed pathogenicity to rice plants. The antibiotics alone controlled sheath blight, BCAs and mutants also controlled the disease effectively. When a BCA or mutant was combined with antibiotic at half the dosage (0.5 x 10^7 CFU mL^1 and 25 ug/mL, respectively), the antibiotic enhanced, weakened, or had no effect on sheath blight control, depending on the compatibility of the BCA and antibiotic.

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