RESEARCH ARTICLE | FEBRUARY 28 2019

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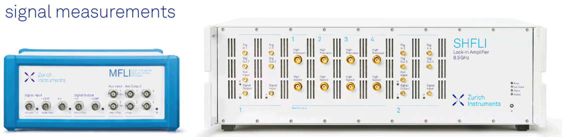
AIP Conf. Proc. 2079, 020019 (2019)
https://doi.org/10.1063/1.5092397



Novel Endophytic Bacteria Isolated from Rice Plant Against Rice Sheath Blight

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Abstract. Endophytic bacteria lived in rice plant have the potential of antagonistic activity against various diseases of rice, including rice sheath blight caused by *Rhizoctonia solani* Kuhn. The goal of the research outlined in this paper was to explore the potential uses of rice endophytic bacteria in control of rice bacterial leaf blight. The antagonistic activity of different bacterial strains were significantly different. The number of endophytic bacteria with relative inhibition ratio (RIR) more than 50 % in first screening was 25, in which there was 16 strains with RIR more than 100 % in first screening. The ratio of strains with significant antagonistic activity was 55.56%. 11 strains with RIR more than 100 % in second screening. RIR of the primary extracellular product of 9 strains was more than 150%.

INTRODUCTION

Endophytic bacterias lived in the healthy plant tissue are widely [1]. There are a variety of groups endophytic bacteria in Rice [2], in which some strains have better effect of prevention and control plant diseases [3].

Rice bacterial leaf blight, caused by *Xanthomonas oryzae* pv. oryzae (Ishiyama) Swings, had been one of the three major diseases in rice, therefore how to effectively control and reduce its damage was the object of a prolonged endeavor of scientific research. Traditionally, selection of resistant cultivars [4] and use of chemical fungicides are the methods to control rice bacterial leaf blight. However, no genetic resources of resistance can protect rice from infection [5], while the repeated uses of chemical bacteriacides caused the development of resistance in bacteria, as well as environmental contamination. Therefore, other manages including biocontrol using the endophytic bacteria have been considered [6-7].

Traditionally, selection of resistant cultivars and use of chemical fungicides are the methods to control rice sheath blight. However, no genetic resources of resistance can protect the vascular system from infection [4], while the repeated uses of chemical fungicides caused the development of resistance in the fungi, as well as environmental contamination. Therefore, other manages including biocontrol using the endophytic bacteria have been considered [7].

In the last decades, endophytic bacteria have been attracted to concerns as novel resource in biocontrol of plants diseases and in promotion of plant growth [6-7]. Endophytes are well adapted to live inside the plants, and they can provide reliable suppression of vascular disease [8] and they do not cause environmental contamination. Endophytes can benefit the host plants by production of phytohormones, fixing nitrogen, sulubilizar phosphate, production of antibiotic compounds, or suppression of phytopathogens by competence of invasion sites etc. [9-11].

There was 45 strains of endophytic bacteria recently isolated from the stems and seeds of rice grown in Jiajiang county and Muchuan county, Sichuan province, China. In this experiment, an investigation about the potential of rice endophytic bacteria in suppression of rice bacterial leaf blight was carried out. The goal of the research outlined in this paper was to explore the potential uses of rice endophytic bacteria in pollution-free prevention and control of rice bacterial leaf blight.

2018 International Conference on Biotechnology and Bioengineering (8th ICBB)
AIP Conf. Proc. 2079, 020019-1–020019-4; https://doi.org/10.1063/1.5092397
Published by AIP Publishing. 978-0-7354-1807-3/$30.00

MATERIALS AND METHODS

Strains

45 strains of endophytic bacteria isolated from the stems and seeds of rice grown in Jiajiang county and Muchuan county, Sichuan province, China recently. Xanthomonas oryzae used in this experiment was isolated from diseased stems of rice grown in Muchuan county, Sichuan province, China.

First Screening of Antagonistic Endophytic Bacteria

An aliquot of 0.1 mL tested bacterial culture (approx.109~1010 cfu ml-1) was spread onto the surface of PDA plate (9 cm in diameter) and 0.1 mL sterilized water instead of bacterial culture was also spread as control. And then, a disc of agar (in diameter of 0.70 mm) with the pathogenic R. solani incubated at 28 °C for 5 days was placed in the center of plate and incubated at 28 °C in dark. After incubation for five days, the size of pathogen colony was measured. All the treatments and control were set in triplicates. In order to quantitatively evaluate the antagonistic activity of the endophytic bacteria, relative inhibition ratio (RIR) was adopted with the following formulae:RIR (%) =(DCK - DT)/ DCK×100%. Where DT is the diameter of pathogen colony in treatment, DCK is the diameter of pathogen colony in control. The isolates with RIR more than 50 % were considered to be significant of antagonistic activity.

Second Screening of Antagonistic Endophytic Bacteria

The strain of endophytic bacteria with RIR more than 50 % in first screening was selected as tested strain in second screening. Agar disc with pathogen was inoculated on PDA plate as described above. The experiment method of second screening was the same way described in first screening. The strain with RIR more than 100 % were considered to be significant of antagonistic activity.

Determination of Antagonistic Activity of Extracellular Products

The tested strain with RIR more than 100 % in second screening was inoculated into 100 mL of NB broth (beef extract 5.0 g/L, Peptone 10.0 g/L, Sodium chloride 5.0 g/L, Agar 15.0 g/L) and incubated with shaking at 180 rpm at 28 °C for 2 days. The culture broth was centrifuged at 12 000 rpm for 10 min and the supernatant was filtered (0.22 μm membrane filter). The obtained culture filtrate was the primary extracellular product and an aliquot of 0.1 mL was spread on the surface of NA agar to test sterility. Determination method of antagonistic activity of the primary extracellular product was the same way described in first screening with the primary extracellular product instead of tested bacterial suspension.

RESULTS AND ANALYSIS

First Screening of Antagonistic Endophytic Bacteria

All strains of 45 endophytic bacteria isolated from rice had antagonistic pathogen activity (Table 1). The antagonistic pathogen activity of different bacterial strains were significantly different. The number of endophytic bacteria with RIR more than 50 % in first screening was 25, in which there was 16 strains (including strain of REB01, REB12, REB16, REB19, REB21, REB24, REB28, REB30, REB32,REB34, REB35, REB39, REB40, REB42, REB44 and REB46) with RIR more than 100 %. There was 9 strains with RIR in the range of 50% to 100% including strain of REB08, REB11, REB14, REB15, REB20, REB22, REB 33, REB 38, and REB41. The ratio of strains with significant of antagonistic activity was 55.56%.

TABLE 1. First screening of antagonistic endophytic bacteria strains isolated from rice against the pathogen of parice sheath blight

Strains	Relative Inhibition Ratio (%)			Average Relative Inhibition Ratio (%)	Strains	Relative Inhibition Ratic (%)			Average Relative Inhibition Ratio (%)
	1	2	3			1	2	3	
REB01	221.7	225.0	220.0	222.2	REB24	188.3	186.7	180.0	185.0
REB02	5.0	8.3	3.3	5.6	REB25	21.7	18.3	25.0	21.7
REB03	33.3	40.0	36.7	36.7	REB26	39.3	45.3	55.0	46.5
REB04	21.7	26.7	18.3	22.2	REB27	38.3	48.3	40.0	42.2
REB05	5.0	13.3	1.7	6.7	REB28	95.0	101.7	105.0	100.6
REB06	33.3	41.7	38.3	37.8	REB29	38.3	36.7	33.3	36.1
REB07	33.3	43.3	31.7	36.1	REB30	188.3	196.7	191.7	192.2
REB08	95.0	80.0	91.7	88.9	REB31	25.0	26.7	36.7	29.4
REB09	28.3	25.0	36.7	30.0	REB32	188.3	186.7	196.7	190.6
REB10	33.3	36.7	43.3	37.8	REB33	91.7	86.7	96.7	91.7
REB11	61.7	68.3	58.3	62.8	REB34	208.3	205.0	218.3	210.6
REB12	133.3	131.7	141.7	135.6	REB35	111.7	108.3	106.7	108.9
REB13	38.3	40.0	40.0	39.4	REB36	5.0	10.0	5.0	6.7
REB14	95.0	86.7	101.7	94.4	REB37	5.0	8.3	3.3	5.6
REB15	95.0	90.0	86.7	90.6	REB38	91.7	93.3	101.7	95.6
REB16	188.3	191.7	203.3	194.4	REB39	208.3	220.0	213.3	213.9
REB17	25.0	25.0	26.7	25.6	REB40	111.7	108.3	118.3	112.8
REB18	38.3	41.7	46.7	42.2	REB41	95.0	91.7	86.7	91.1
REB19	111.7	108.3	118.3	112.8	REB42	188.3	186.7	180.0	185.0
REB20	91.7	93.3	101.7	95.6	REB43	21.7	18.3	25.0	21.7
REB21	208.3	220.0	213.3	213.9	REB44	95.0	101.7	105.0	100.6
REB22	95.0	91.7	86.7	91.1	REB45	38.3	36.7	33.3	36.1
REB23	5.0	8.3	3.3	5.5	REB46	188.3	196.7	191.7	192.2

Second Screening of Antagonistic Endophytic Bacteria

All strains of 25 endophytic bacteria with RIR more than 50 % had antagonistic pathogen activity (Table 2). The antagonistic activity of different bacterial strains were significantly different. There was 11 strains (including strain of REB01, REB16, REB21, REB24, REB30, REB32, REB34, REB39, REB40, REB42 and REB46) with RIR more than 100 %.

TABLE 2. Second screening of antagonistic endophytic bacteria strains isolated from rice against the pathogen of parice sheath blight

Strains	Relative Inhibition Ratio (%)			Average Relative Inhibition Ratio (%)	Strains	Relative Inhibition Ratio (%)			Average Relative Inhibition Ratio (%)
	1	2	3			1	2	3	
REB01	220.0	215.0	222.5	219.2	REB30	220.0	215.0	218.0	217.7
REB08	78.0	92.0	98.0	89.3	REB32	195.0	192.0	191.5	192.8
REB11	70.0	75.7	83.7	76.5	REB33	98.0	95.3	95.0	96.1
REB12	91.0	81.0	84.0	85.3	REB34	205.0	205.0	208.0	206.0
REB14	95.0	98.0	96.0	96.3	REB35	92.0	91.7	99.0	94.2
REB15	90.0	85.0	95.0	90.0	REB38	95.0	92.0	91.5	92.8
REB16	180.0	185.7	193.7	186.5	REB39	194.0	195.0	192.0	193.7
REB19	90.0	85.0	95.0	90.0	REB40	128.0	139.0	141.7	136.2
REB20	90.0	85.0	94.7	89.9	REB41	85.0	85.0	88.0	86.0
REB21	210.0	205.0	208.3	207.8	REB42	190.0	185.0	194.7	189.9
REB22	95.0	98.0	96.0	96.3	REB44	90.0	95.0	98.3	97.8
REB24	178.0	189.0	193.0	186.7	REB46	198.0	195.3	195.0	196.1
REB28	95.0	93.3	99.0	95.8					

Determination of Antagonistic Activity of Extracellular Products

Antagonistic activity of the primary extracellular product of 11 endophytic bacteria strain with more than 100 % in second screening was relatively high (Table 3). Antagonistic activity of the primary extracellular product of all tested strains was lower than of same tested strains. RIR of the primary extracellular product of all tested strains except strain REB16 and REB40 was more than 150%.

TABLE 3. Antagonistic activity of extracellular products of endophytic bacteria with RIR more than 100%

Strains	Relative Inhibition Ratio (%)			Average Relative Inhibition Ratio (%)	Strains	Relative Inhibition Ratio (%)			Average Relative Inhibition Ratio (%)
	1	2	3			1	2	3	
REB01	215.0	210.0	204.0	209.7	REB34	178.0	172.0	182.0	177.3
REB16	128.0	131.7	153.3	134.3	REB39	150.0	156.0	163.0	156.3
REB21	195.0	193.0	192.0	193.3	REB40	135.0	124.3	117.7	125.7
REB24	205.0	194.3	187.7	195.7	REB42	195.0	185.0	169.0	183.0
REB30	205.0	195.0	185.0	195.0	REB46	178.0	182.0	193.0	184.3
REB32	148.0	152.0	163.0	154.3					

CONCLUSION

A total of 45 isolates were isolated from the stems and seeds of rice grown in Jiajiang county and Muchuan county, Sichuan province, China. All tested strains had antagonistic pathogen activity against to R. solani. There was 25 strains endophytic bacteria with RIR more than 50 % in first screening. The antagonistic pathogen activity of different bacterial strains were significantly different. There was 11 strains with RIR more than 100 % in second screening. RIR of the primary extracellular product of 9 tested strains was more than 150%.

At present, the antagonistic pathogen substance were screened based on vitro anti method from herbs and their extracts, in which endophytic bacteria strains isolated from rice showed better antagonistic activity [12]. In this experiment, 45 isolates isolated from the stems and seeds of healthy rice had antagonistic pathogen activity against to *Rhizoctonia solani*, pathogen of rice sheath blight. Antagonistic activity of the primary extracellular product of 11 endophytic bacteria strain with more than 100 % in second screening was relatively high. Therefore, Screening of antagonistic substances to control rice sheath blight from antagonistic endophytic bacteria had prospects for development.

ACKNOWLEDGMENTS

The work was supported by the scientific research fund of the Sichuan provincial education department (Grant NO: 15TD0026), and Key Research Project of Leshan Science and Technology Bureau (Grant NO: 18ZDYJ0097).

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