SCREENING *BACILLUS* STRAINS FOR ANTAGONISTIC ACTIVITY AGAINST *FUSARIUM* SP. AND *PHYTOPHTHORA PALMIVORA* CAUSING DISEASES IN CORN (*ZEA MAYS* L.)

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ABSTRACT

61 Bacillus strains originally isolated from Can Gio manglicolous soil were evaluated in vitro for possible antagonistic activity against Fusarium sp. *and Phytophthora palmivora. All of screened strains possessed antagonistic properties. Isolates B44 was the most effective in inhibiting Fusarium* sp. *growth. Isolates B34 was the most effective in inhibiting P. palmivora growth. These two isolates were tested in vivo for controlling diseases in corn* (*Zea mays* L.). *Only B44 strains could efficiently reduce corn death rate.*

Keywords: antagonistic *Bacillus.*

TÓM TẮT

***Tuyển chọn chủng Bacillus có khả năng kháng Fusarium* sp. và Phytophthora palmivora gây bệnh trên cây bắp (*Zea mays* L.)**

61 chủng vi khuẩn Bacillus có nguồn gốc từ rừng ngập mặn Cần Giờ được kiểm tra hoạt tính đối kháng với nấm Fusarium sp. *và Phytophthora palmivora. Tất cả các chủng được kiểm tra đều đối kháng với 2 loại nấm gây bệnh trên ở các mức độ khác nhau. Trong đó, chủng B44 kháng Fusarium* sp. *mạnh nhất, chủng B34 có khả năng kháng Phytophthora palmivora tốt nhất. Hai chủng này được khảo sát khả năng kháng bệnh do Fusarium* sp. *và P. palmivora gây ra trên cây bắp. Kết quả cho thấy chỉ có chủng B44 có khả năng kiểm soát tốt bệnh do Fusarium* sp.

Từ khóa: *Bacillus* đối kháng.

1. Introduction

The genus *Fusarium* includes many species that cause plant diseases, such as vascular wilts, root, stalk and cob rots, collar rot of seedlings, and rots of tubers, bulbs and corms. Cob rots in maize, caused mainly by *F. graminearum* and *F. verticillioides*, are becoming increasingly important in Vietnam. Both species produce mycotoxins which contaminate the grain [4]. *Phytophthora* species attack a wide range of plants, and are responsible for some of the world's most destructive plant diseases [1]. The genus *Phytophthora* is responsible for extensive economic damage in a wide range of different crops throughout the country, including fruit, vegetables, tree plantations and other agricultural crops in Vietnam. [1]

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Plant diseases need to be controlled to maintain the quality and abundance of food, feed, and fiber produced by growers around the world. Different approaches may be used to prevent, mitigate or control plant diseases. Beyond good agronomic and horticultural practices, growers often rely heavily on chemical fertilizers and pesticides. Such inputs to agriculture have contributed significantly to the spectacular improvements in crop productivity and quality over the past 100 years. However, the environmental pollution caused by excessive use and misuse of agrochemicals, as well as fear-mongering by some opponents of pesticides, hasled to considerable changes in people's attitudes towards the use of pesticides in agriculture. Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market. Additionally, the spread of plant diseases in natural ecosystems may preclude successful application of chemicals, because of the scale to which such applications might have to be applied. Consequently, some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases. Among these alternatives are those referred to as biological controls. [5]

Many microorganisms with antagonistic properties have been identified, evaluated and registered for commercial use such as *Bacillus subtilis* "Avogreen", registered in South Africa by the University of Pretoria, for the control of avocado fruit diseases, and the yeast "Aspire" registered for control of citrus mold and marketed by Ecogen Inc. in the USA. However, there is obviously an untapped pool of microorganisms of which many more beneficial microorganisms are yet to be discovered. The search for new microorganisms with antagonistic properties is therefore a continuous process.

Bacillus species produce spores that are resistant to desiccation, heat, UV irradiation, and organic solvents. These qualities make them more resistant to adverse weather conditions. The antagonistic activity of *Bacillus* species against many pathogens has been demonstrated [3].

2. Materials and methods

Microbial strains

Bacillus strains were previously isolated from soil samples taken in Can Gio mangrove, Ho Chi Minh City, Vietnam.

Fusarium sp., *Phytophthora palmivora* were obtained from the collection of Nong Lam Unviversity, Ho Chi Minh City.

In vitro screening of isolates for antagonism

Bacillus isolates were screened *in vitro* against *Fusarium* sp. and *Phytophthora palmivora* by applying a dual culture technique in 9-cm Petri dishes on PDA medium. *Bacillus* isolates were streaked across the centre of the Petri dish. Two discs 5 mm in diameter cut from a 3 day-old culture of pathogenic fungi were placed at each side of

the antagonist. The distance between the two microorganisms was 2.5 cm. Dishes were incubated at room temperature for 5 days. Percent growth inhibition of pathogenic fungi after 5 days was calculated by the formula of Whipps (1987): (R1-R2)/R1*100, where R1 is the fungal radial growth in the direction opposite to the antagonist and R2 is the radial growth toward the antagonist. Growth inhibition was measured on a scale from 0 to 4 (Korsten et al, 1995), where 0 = no growth inhibition, 1 = 1 to 25% growth inhibition, 2 = 26 to 50% growth inhibition, 3 = 51 to 75% growth inhibition, 4 = 76 to 100% growth inhibition. All *in vitro* antagonism assays were made in triplicate.

***In vivo* pot experiments**

To test the suppression of corn diseases caused by *Fusarium* sp. and *Phytophthora palmivora*, 10^5 CFU/mL of pathogenic fungi spores in 5 mL of sterile water were treated to soil in pots. The corn seeds were pre-germinated for three days in Petri dishes containing sterile distilled water. After 2 days of the pathogenic spores treatment, 3 day-old corn seedlings were transplanted to these pots. Then 100 mL *Bacillus* cell suspension (10^8 CFU/mL) were treated to the pots. The control pots were received only sterile water. All pots were checked every day for signs of infection. Each experiment included 10 plants per treatment with three replications. Survivals were counted 2 weeks after the introduction of *Bacillus* cell suspension.

3. Results

3.1. In vitro screening of isolates for antagonism against Fusarium sp.

61 *Bacillus* isolates were screened for antagonistic activity again *Fusarium* sp. All of these isolates were found to be antagonist bacteria against tested fungi. Among them, 9 isolates made the clear inhibition zone as mentioned in table 3.1.

Table 3.1. *Antagonism of Bacillus isolates against Fusarium* sp.

No.	Strains	Percent growth inhibition (%)	The size of the growth inhibition zone (cm)
1	B3	26.25 ± 3.98	0.27 ± 0.06
2	B4	11.67 ± 2.14	0.22 ± 0.04
3	B16	22.09 ± 6.30	0.43 ± 0.12
4	B17	20.00 ± 5.50	0.52 ± 0.12
5	B18	28.33 ± 13.24	0.38 ± 0.15
6	B21	28.75 ± 4.32	0.48 ± 0.10
7	**B44**	**29.17 ± 1.35**	**0.71 ± 0.06**
8	B53	14.58 ± 5.61	0.22 ± 0.03
9	B60	18.75 ± 9.92	0.21 ± 0.02

According to Korten et al. scale (1995), 5 isolates (B4, B16, B17, B53 and B60) inhibited *Fusarium* sp. race 1, 4 isolates (B3, B18, B21 and B44) gave more than 25% inhibition and belonged to inhibition categories 2. Isolate B44 was the most effective antagonist *in vitro* and caused 29.17% growth inhibition.

Other 52 isolates grew over the fungal mycelial surface and multiplied extensively on it. Among them, 3 isolates (B10, B23 and B24) were the most effective antagonists.

Basing on their antagonistic efficiency against *Fusarium* sp., isolate B44 was selected for screening under field conditions.

Figure 3.1. *Antagonism of Bacillus B44 against Fusarium* sp.

Figure 3.2. *Fusarium* sp. *grown on PDA (control)*

3.2. In vitro screening of isolates for antagonism against Phytophthora palmivora

40 *Bacillus* isolates were screened for antagonistic activity again *Phytophthora palmivora*. All of these isolates were found to be antagonistic bacteria against tested fungi. Among them, 37 isolates made the clear inhibition zone as mentioned in table 3.2.

Table 3.2. *Antagonism of Bacillus isolates against Phytophthora palmivora*

No.	Strains	Percent growth inhibition (%)	The size of the growth inhibition zone (cm)
1	B1	53.30 ± 5.20	0.37 ± 0.20
2	B2	54.20 ± 9.20	0.08 ± 0.14
3	B3	46.70 ± 3.60	0.23 ± 0.27
4	B4	56.70 ± 6.10	0.84 ± 0.24
5	B6	36.30 ± 19.60	0.32 ± 0.34
6	B7	62.10 ± 9.40	0.11 ± 0.13
7	B8	55.40 ± 6.30	0.27 ± 0.17
8	B10	47.50 ± 12.40	0.36 ± 0.38
9	B13	65.00 ± 6.80	0.60 ± 0.13

10	B14	80.00 ± 1.70	1.06 ± 0.10
11	B15	51.70 ± 16.50	0.11 ± 0.19
12	B17	60.80 ± 6.80	1.29 ± 0.08
13	B18	68.80 ± 6.20	0.41 ± 0.11
14	B19	73.00 ± 3.40	0.70 ± 0.29
15	B21	65.80 ± 5.40	1.00 ± 0.46
16	B22	57.50 ± 7.90	0.95 ± 0.54
17	B24	57.50 ± 12.00	0.62 ± 0.21
18	B25	58.30 ± 8.10	0.58 ± 0.22
19	B26	59.60 ± 3.90	0.50 ± 0.16
20	B27	62.90 ± 2.00	0.47 ± 0.13
21	B29	60.00 ± 14.10	0.18 ± 0.19
22	B30	58.30 ± 5.70	0.79 ± 0.19
23	B31	58.30 ± 7.00	0.63 ± 0.10
24	B32	61.70 ± 3.20	0.75 ± 0.23
25	B33	62.90 ± 2.60	0.43 ± 0.42
26	**B34**	**81.30 ± 6.20**	**1.17 ± 0.15**
27	B36	69.60 ± 7.50	0.85 ± 0.27
28	B38	77.10 ± 6.90	0.92 ± 0.47
29	B45	54.50 ± 3.90	0.60 ± 0.23
30	B46	58.30 ± 4.60	0.97 ± 0.31
31	B47	55.40 ± 5.60	0.80 ± 0.31
32	B48	60.80 ± 7.90	0.38 ± 0.27
33	B50	56.30 ± 7.20	0.58 ± 0.28
34	B52	42.50 ± 10.80	0.45 ± 0.25
35	B56	54.60 ± 4.20	1.04 ± 0.16
36	B57	57.90 ± 6.10	0.83 ± 0.19
37	B61	46.30 ± 5.40	0.44 ± 0.23

According to Korten et al. scale (1995), 4 isolates inhibited *P. palmivora* race 2, 30 isolates inhibited tested fungi race 3, 3 isolates (B14, B34, B38) gave more than 75% inhibition and belonged to inhibition categories 4. Isolate B34 was the most effective antagonist and caused 81.30 % growth inhibition.

Other 3 isolates didn't make the clear inhibition zones but they grew much faster than tested fungi and occupied nearly all the plate surface. They competed nutrition and habitat with the fungi so that they could control the growth of the tested fungi.

On the basis of *in vitro* performance, isolate B34 was selected for screening under field conditions.

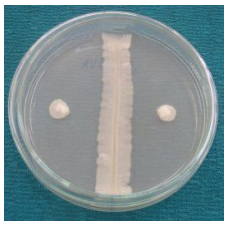

Figure 3.3. *Antagonism of Bacillus B34 against P. palmivora*

Figure 3.4. *P. palmivoragrown on PDA medium (control)*

3.3. In vivo pot experiments

3.2.1. In vivo pot experiments for inhibition of wilt of corn trees caused by Fusarium sp.

Table 3.3. *Effect of Bacillus B44 of inhibition of wilt of corn trees caused by Fusarium* sp.

	Normal	**Treatment**	**Control**
Alive trees	17	20	5
Alive rate (%)	57	67	17
Death trees	13	10	25
Death rate (%)	43	33	83

These tests showed that there was more disease (83% of plants wilted) in controls than in the trials (33% of plants wilted). All corn trees in controls died in the next 2 days whileas those in trials were still alive heathily. Treatment of soil with *Bacillus* B44 significantly reduce death rate of corn.

		
Normal	Treatment (*Fusarium* sp. + B44)	Control (*Fusarium* sp. only)
Figure 3.5. *Fusarium* sp.		

3.2.1. In vivo pot experiments for inhibition of wilt of corn trees caused by Phytophthora palmivora

Table 3.3. *Effect of Bacillus B44 of inhibition of wilt of corn trees caused by Phytophthora palmivora*

	Normal	**Treatment**	**Control**
Alive trees	25	15	7
Alive rate (%)	83%	50%	23%
Death trees	5	15	23
Death rate (%)	17%	50%	77%

These tests showed that there was a high incident of wilt in both trial and positive control pots. Although there was more disease (77% of plants wilted) in positive control than in the trials (50% of plants wilted), all trees in both treatments grew weakly and died in the next few days. Isolates B34 were not effective in controlling disease in corn caused by *P. palmivora.*

		
Normal	Treatment (*P. palmivora* + B34)	Positive control (*P. palmivora* only)
Figure 3.6. *Efficency of Bacillus B34 for in vivo inhibition of wilt caused by P. palmivora*		

Results from the dual culture assay together with *in vivo* experiments showed that isolate B44 was finally selected as an antagonistic *Bacillus* sp. with potential for use in control of diseases caused by *Fusarium* sp. in corn trees.

In conclusion, the isolate B44 preliminary identified as *Bacillus* sp. showed antagonistic activities under laboratory, against *Fusarium* sp.. These activities may be due to enzymatic activities and/or other yet non determined metabolites produced by strains which are highly involved in biocontrol. Based on our knowledge of key characteristics of *Bacillus* antagonistic isolates, along with the known mechanisms of *Bacillus* antagonism toward fungi, we were better able to direct suggest that the isolate B44 can be exploited as a biological control agent candidate. Prior to this, additional experiments such as molecular identification and biochemical tests need to be carried out to completely characterize this isolate and determine their ability to show some of the major traits involved in the biocontrol of phytopathogens.

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