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Mycelial growth and sporulation of *Emericella nidulans*, a new fungal antagonist on two culture media

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Potato dextrose broth produced significantly ($p< 0.01$) heavier fresh and dried mycelial pellets as compared to coconut water dextrose broth. Moreover, fresh and dried weights of mycelia were significantly ($p<0.01$) influenced by pH of the media. Highest fresh and dried mycelial pellets were obtained in pH 5 while lowest weight of mycelial pellets was obtained in pH 8. *E. nidulans* significantly grew faster and produced more spores in coconut water dextrose agar. Slow mycelial growth, narrower mycelial diameter and lesser number of spores were observed in *E. nidulans* grown in potato dextrose agar. Coconut water dextrose agar at pH 6 registered significantly highest mycelial growth and spore formation throughout the observation period while those cultured in potato dextrose agar at pH 5 gave the lowest mycelial growth ($p<0.01$).

Key words: *Emericella nidulans*, fungal antagonist, coconut dextrose medium

Introduction

Due to the adverse effect of chemical pesticides on animals, humans and ecosystem, there is increasing interests on searching for alternative methods of controlling plants diseases. As a result, several pest management strategies such as cultural, physical, biological methods and use of resistant varieties have been tried. Recently, some new species of promising fungal antagonists have been discovered which can be used to control plant diseases. Bioactive compounds extracted from several species of fungi were reported to inhibit the growth of many plant pathogenic fungi (Kanokmedhakul *et al.*, 2006; Thongsri and Soytong, 2004; Srinon *et al*., 2004; Suwannapong and Soytong, 2002). For

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instance, the bioactive compound Tricotoxin A50 was extracted from *Trichoderma harzianum* PC01; and Chaetoglobosin C was extracted from *Chaetomium globosum.* These compounds have been reported to elicit resistance or immunity in plants by inducing oxidative burst in plant cells (Nuchdonrong *et al.*, 2004).

Among the different species isolated *Emerecilla nidulans* is one of the promising fungal antagonists that can be used to control the fungal wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. The suitable media and other factors for optimum mycelial growth and proliferation and spore production of this organism have not been fully established.

Materials and methods

The promising antagonistic fungus, *Emericella nidulans* strain EN was obtained from the Laboratory of Dr. Kasem Soytong. This was transferred onto PDA plates, and incubated at room temperature. The morphological characteristics of the fungus were studied under compound microscope.

Growth performance of E. nidulans in solid media of varying pH levels

Potato dextrose agar (PDA) was prepared by boiling 200 g potato, 20 g dextrose and 20 g agar in 1000 ml of water. Coconut water dextrose agar (CWDA) was prepared by boiling 20 g dextrose and 20 g agar in 1000 ml of coconut water. Media were separately prepared and their pH levels were adjusted with either hydrochloric acid (HCl) or sodium hydroxide (NaOH) to get the required pH level. The pH of the media was measured using electrical pH meter. The pH of the media was set before sterilization in autoclave at 121^{o}C, 15 psi for 20 min. Thereafter, the media were warmed to 50^{o}C and 15 ml was poured into each sterilized petri dish (9 cm diameter).

Inoculation

Agar plugs of *E. nidulans* cultured on PDA were taken by cutting the peripheral colony with sterilized cork borer (approximately 0.3 cm). The culture agar plug was transferred in the middle of each treatment plate with solid media at different pH levels. The inoculated plates were incubated and kept at room temperature at approximately 27 to 30^{o}C for 7 days. Colony diameter (cm) was measured and the number of spores was determined using haemocytometer. With this, the colony in each treatment plate was gently removed and aseptically transferred into 10 ml sterilized water in each test tube. A drop of this solution was placed into the haemocytometer and was

mounted for counting under the compound microscope. The number of spores per ml was computed using the formula below:

Number of spore per ml = number of spores in four corner and center squares x 25 x 10^4.

The experiment was set up in 2 x 4 factorial experiments in Completely Randomized Design (CRD). Each treatment was replicated four times with three plates per replication. The following are the different treatments evaluated in this study: factor A was kinds of media where A1 = PDA (Potato Dextrose Agar) and A2 = CWDA (Coconut Water Dextrose Agar). Factor B was pH levels where B1= 5, B2=6, B3=7 and B4 = 8.

Mycelial growth of Emericella nidulans in liquid media of varying pH levels

Potato dextrose broth (PDB) was prepared by boiling 200 g of potato and 20 g of dextrose in 1000 ml of water. Coconut water dextrose broth (CWDB) was prepared by boiling 1000 ml of coconut water and 20 g dextrose. Twenty ml of each medium was separately placed in flask. The pH was adjusted by adding either HCl or NaOH to get the required pH value. Media were sterilized in autoclave at 121^{o}C, 15 psi for 20 minutes.

Inoculation

An agar plug of *E. nidulans* was transferred into each flask. The inoculated flasks were incubated in electrical shaker for 7 days at room temperature approximately 27 to 30^{o}C. After seven days, the culture on each flask was separately filtered using Whatman filter paper No. 4 to get the fresh mycelial pellets. The pellets were air dried at room temperature for 24 hours. Fresh and dried mycelia pellets were weighed (g) using electrical balance.

Results and discussion

Fresh and dried weight of mycelial pellets of Emericella nidulans grown in liquid media

E. nidulans strain EN was cultured on potato dextrose broth (PDB) and coconut water dextrose broth and incubated on electrical shaker at room temperature (approx. 27-30^{o}C) for 15 days at pH levels 5, 6, 7 and 8 (Fig. 1 and 2). Results showed that *E. nidulans* grown in potato dextrose broth produced significantly heavier fresh (Fig. 3) and dried mycelial pellets (Fig. 4) while those cultured in coconut water dextrose broth yielded lower fresh and dried mycelial pellets.

Coconut Water Dextrose Broth

pH5 **pH6** **pH7** **pH 8**

Fig. 1. Mycelial pellets *of Emericella nidulans* on coconut dextrose broth.

Potato Dextrose Broth

pH5 **pH6** **pH7** **pH 8**

Fig. 2. Mycelial pellets *of Emericella nidulans* on potato dextrose broth.

This result suggests that potato dextrose broth is a more suitable medium for mycelial production of *E. nidulans* than coconut water dextrose broth. This conforms to the general observation that most fungi thrive in potato dextrose medium which supported mycelial growth and proliferation. Meanwhile, the pH of the culture media significantly influenced the production of mycelial pellets. Highest fresh and dried mycelial pellets were obtained in broth with pH 5 with mean values of 18.64 g and 1.18 g, respectively. The fresh and dried weights of mycelial pellets formed in culture broth with pH 6, 7 and 8 were comparable. This result indicates that pH 5 is the most suitable pH which favors the production of mycelial pellets. Carlile and Watkinson (1994) reported that fungi can tolerate a wide range of pH, but most media used to culture fungi are acidic. Most fungi grew well over the range of pH 3 to 7.

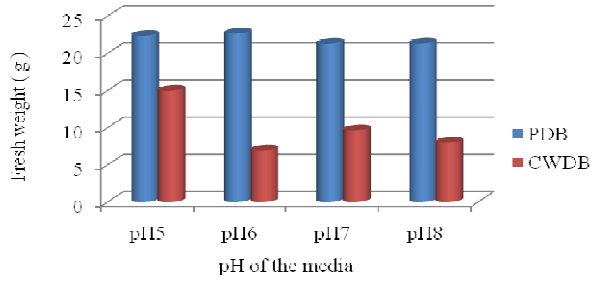

Fig. 3. Mean fresh weight of mycelial pellets (g).

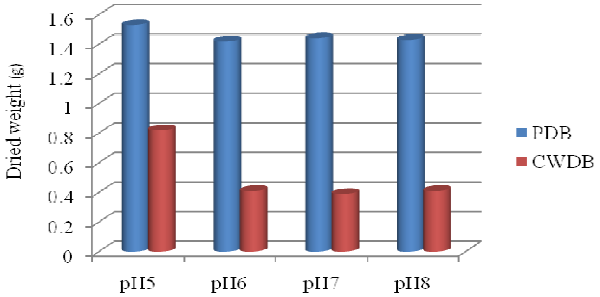

Fig. 4. Mean dried weight of mycelial pellets (g).

Growth performance of Emericella nidulans in solid media of varying pH levels

The mycelia diameter and spore production of *E. nidulans* in coconut water dextrose agar (CWDA) and potato dextrose agar (PDA) at varying pH levels are presented in Table 1 and Fig. 5. Among the treatment combinations, coconut water at pH 6 registered the highest mycelial growth and spore formation throughout the observation period while those cultured in potato dextrose agar at pH 5 gave the lowest mycelial growth. Results showed that at each observation period (4 days, 8 days and 12 days), *E. nidulans* cultured in coconut dextrose water agar at different pH levels were different from each other. This result indicates that the pH of this media could affect the mycelial growth of *E. nidulans*. Slow mycelial growth, narrower mycelial diameter and lesser number of spores were observed in *E. nidulans* grown in potato dextrose agar (Fig. 3 and 4). This result indicates that coconut water dextrose agar is more suitable for the mycelial growth and spore production of *E. nidulans*. This result confirms previous reports that coconut water is an excellent medium for

mycelial ramification of mushrooms and other microscopic fungi (Garcia *et al.*, 2004 and Tayamen *et al.*, 2004). Meanwhile, after four days of incubation, *E nidulans* cultured at pH 6 yielded lowest mycelial diameter (Table 1). Throughout the incubation period, the maximal mycelial growth was observed in medium with pH 8; however, significant difference in mycelial growth was noted during the 12th day of incubation. The mycelial growth in pH 5, 7 and 8 are comparable but significantly different from pH 6.

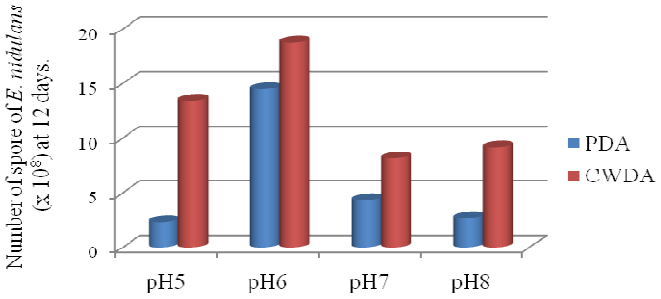

Fig. 5. Mean number of spore of *Emericella nidulans* (x 10^8) in PDA and CWDA at different pH levels.

Conclusion

Potato dextrose broth at pH 5 favors the production of mycelial pellets of *E. nidulans*, while coconut water dextrose agar at pH 6 is suitable for spore production.

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Table 1. Mean colony diameter of *Emericella nidulans* from solid media and different pH levels.

Media	Colony diameter (cm)													
	4 days				Mean	8 days				Mean	12 days				Mean
	5	6	7	8		5	6	7	8		5	6	7	8	
PDA	2.5	1.62	2.47	2.63	2.28b	5.15	3.00	5.05	5.07	4.57b	7.65	6.13	7.17	7.29	7.06b
CDA	3.20	3.68	3.25	3.52	3.41a	6.23	7.20	6.70	7.20	6.83a	8.73	8.92	8.90	8.90	8.86a
Mean	2.85	2.65	2.86	3.08		5.69	5.10	5.88	6.14		8.19a	7.52b	8.03a	8.09a	

Mean with the same superscript are not significantly different from each other at 5% level of significance**.**