African Journal of Microbiology Research Vol. 5(17), pp. 2451-2454, 9 September, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR11.078
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

Effect of boric acid on the growth and production of β-glucosidase in *Paecilomyces variotii*

Swi See Ang[1], Abu Bakar Salleh[1,2]* and Fatimah Abu Bakar[1,3], Nor Azah Yusof[1,4], Muhammad Zukhrufuz Zaman[3], and Lee Yook Heng[5]

[1]Laboratory of Industrial Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor D.E., Malaysia.
[2]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, UPM Selangor D.E., Malaysia.
[3]Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor D.E., Malaysia.
[4]Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor D.E., Malaysia.
[5]School of Chemical Sciences and Food Technology, Faculty of Science and and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor D.E., Malaysia.

Accepted 30 July, 2011

Boric acid was examined for antifungal activity against several species of fungi. The growth and production of β-glucosidase in several strains of fungi were tested in the media containing various concentrations of boric acid. The results obtained indicate that the growth of *Trichoderma* strains was reduced with increasing amount of boric acid up to 0.3% (w/v). However, *Paecilomyces variotii* was able to tolerate boric acid above 0.3% (w/v) by increasing its growth. *Trichoderma* also showed low production of β-glucosidase as compared to *P. variotii*. Present study revealed that boric acid works as effective fungicide by inhibiting the production of β-glucosidase and the growth of fungi. This suggests that boric acid might be useful for the treatment of fungal infection caused by *P. variotii* or *Trichoderma* strains.

Key words: Growth, β-glucosidase, boric acid, inhibition, *Paecilomyces variotii*.

INTRODUCTION

Paecilomyces is a filamentous fungus which inhabits soil, decays plants and grows as a surface mold on live and dead wood (Abbas et al., 2009; Parker et al., 1999). *Paecilomyces* can cause various infections in human and the diseases are known as paecilomycosis (Salle et al., 2005). In addition, this type of fungi may also cause allergic disorder. However, due to the limited data of anti-fungal agent against *Paecilomyces*, the guideline of treatment for *Paecilomyces* is not well established (Salle et al., 2005). *Paecilomyces variotii* is one of the most common fungi belonging to the genus of *Paecilomyces* (Tarkkanen et al., 2004) which can be found in air and food (Houbraken et al., 2010). As other species in this genus, *P. variotii* is also associated with human infection incidences, for instances, kreatitis, corneal ulcer and endophthalmitis (Abbas et al., 2009; Anita et al., 2010).

Boric acid (H_3BO_3) is a boron compound which exhibits antimicrobial properties (Lueck, 1980). This chemical has been used as a preservative in various fields such as lumber, baggase and food products as well as for medication purposes (De Seta et al., 2009). Due to its antimicrobial properties, the usage of boric acid as pesticide has been known to be useful to kill several types of pest such as fleas, cockroaches, wood-

*Corresponding author. E-mail. abubakar@biotech.upm.edu.my. Tel: +603-8946 6695. Fax: +603-8943 0913.

destroying insects (including termites) and fungi (See et al., 2010, Strong et al., 2001). Generally, boric acid is considered to be a potential therapy for infection disease (De seta et al., 2009). Therefore, this study was subjected to determine the effect of boric acid on mycelia growth of *P. variotii* and other fungal species. In addition, the production of β-glucosidase by *P. variotii* was also observed.

MATERIALS AND METHODS

Fungal species

Fungi *P. variotii* (ATCC 62398), *Trichoderma sp.* (Department of Biochemistry, UPM), *Trichoderma atroviride, Trichoderma harzanium* I26, and *Trichoderma harzanium* I38 (Department of Biology, UPM) were used in this research.

Growth assessment

Growth assessment was carried out by cultivating the fungi in potato dextrose agar (PDA) containing 0 to 0.3% (w/v) boric acid. For inoculation, a sterile metal cork borer was used to cut a 1 cm diameter of mycelia disks from the periphery of a PDA cultures. It was then aseptically inoculated inversely onto a centre of the solid medium plate. The inoculated plates were incubated at 30°C until 15 days. The mycelium of each plate was measured by the diameter of mycelia's growth and the reading was taken every day. The percentage of standard petri dish covered was then calculated.

β-glucosidase production

Studies of each strain of fungi were also carried out for their ability to produce β-glucosidase in liquid medium containing 0.1% (w/v) boric acid. After that, the most resistance microorganism was chosen to study the production of β-glucosidase in medium containing 0.1, 0.5, 1.0 and 1.5% (w/v) boric acid. The concentration of β-glucosidase was determined by using 4-nitrophenyl-β-d-glucoside (NPG) as a substrate.

RESULTS AND DISCUSSION

Figure 1 shows the effect of different concentrations of boric acid on the growth of tested fungal species. *Trichoderma* strains exhibit similar growth pattern during 15 days of screening study. They covered the whole surface of the Petri dish containing 0 to 0.2% (w/v) boric acid. However, in the medium containing 0.3% (w/v) boric acid and above, no growth or only slightly growth was observed. *Trichoderma* strains were able to grow in the media with boric acid concentration of up to 0.3% (w/v). In contrast, *P. variotii* was able to survive in the boric acid concentration at above 0.3% (w/v). A study from Parker et al. (1999) stated that the survival of *P. variotii* in the presence of boric acid was related to the production of β-glucosidase in order to increase the glucose supply from the carbon source in the medium. Boric acid readily forms complex with hydroxyl groups such as glucose (Strong et al., 2001), and this restricts boric acid from being absorbed by the fungi. Therefore, the fungi continue to grow as boric acid has less effect on this fungus. However, the exact and specific process for increasing of the β-glucosidase activity in *P. variotii* was still unknown (Parker et al., 1999). Hence, further experiment was carried out in order to study the effect of boric acid on the production of β-glucosidase.

Figure 2 indicates the production of β-glucosidase of several species of fungi in the presence of 0.1% (w/v) boric acid. The result displays that β-glucosidase level of *P. variotii* increased much higher than *Trichoderma* strains during ten days of incubation. This will lead to different growth ability between each fungal species. Furthermore, due to low production of β-glucosidase, the growth of *Trichoderma* was suppressed. In other word, *Trichoderma* strains were more susceptible to boric acid than *P. variotii*. As such, boric acid has strong fungicidal effect towards *Trichoderma* strains.

Apart from that, *P. variotii* was selected to study the effect of 0.1 to 1.5% (w/v) boric acid on the production of β-glucosidase over ten days of incubation. As shown in Figure 3, the production of β-glucosidase by *P. variotii* decreased in accordance with the increment of boric acid concentration. This was due to the inhibitory effect of boron compound which acts as fungicide (Cox, 2004; Strong et al., 2001). This study showed that although *P. variotii* could survive better than other microorganisms in boron environment, boric acid is still powerful in inhibiting its growth in high amount. In addition, based on a study by Hamilton and Wolf (2007), boric acid is considered as an effective fungicide in the concentration of 8% and above. Boron compound halts the production of fungi spore and this leads to the suppression of fungus growth (Cox, 2004).

Conclusion

Present study is essential for understanding the growth of *P. variotii* and several strains of *Trichoderma* in the presence of boric acid. Boric acid exhibited fungicidal effect against several strains of fungi. This recommends that boric acid has the potential to be used as a treatment to control fungi. Nevertheless, the ability of fungi to produce β-glucosidase in boron environment enables them to tolerate certain concentrations of boric acid. Since that *P. variotii* possessing this unique trait, it could be used as a microbial agent in constructing bioassay system to determine boric acid in future research.

ACKNOWLEDGEMENT

The authors are grateful to the Ministry of Science, Technology and Innovation, Malaysia through the Malaysian Genome Institute (MGI) for financial support (Research grant no. 001-002-0027).

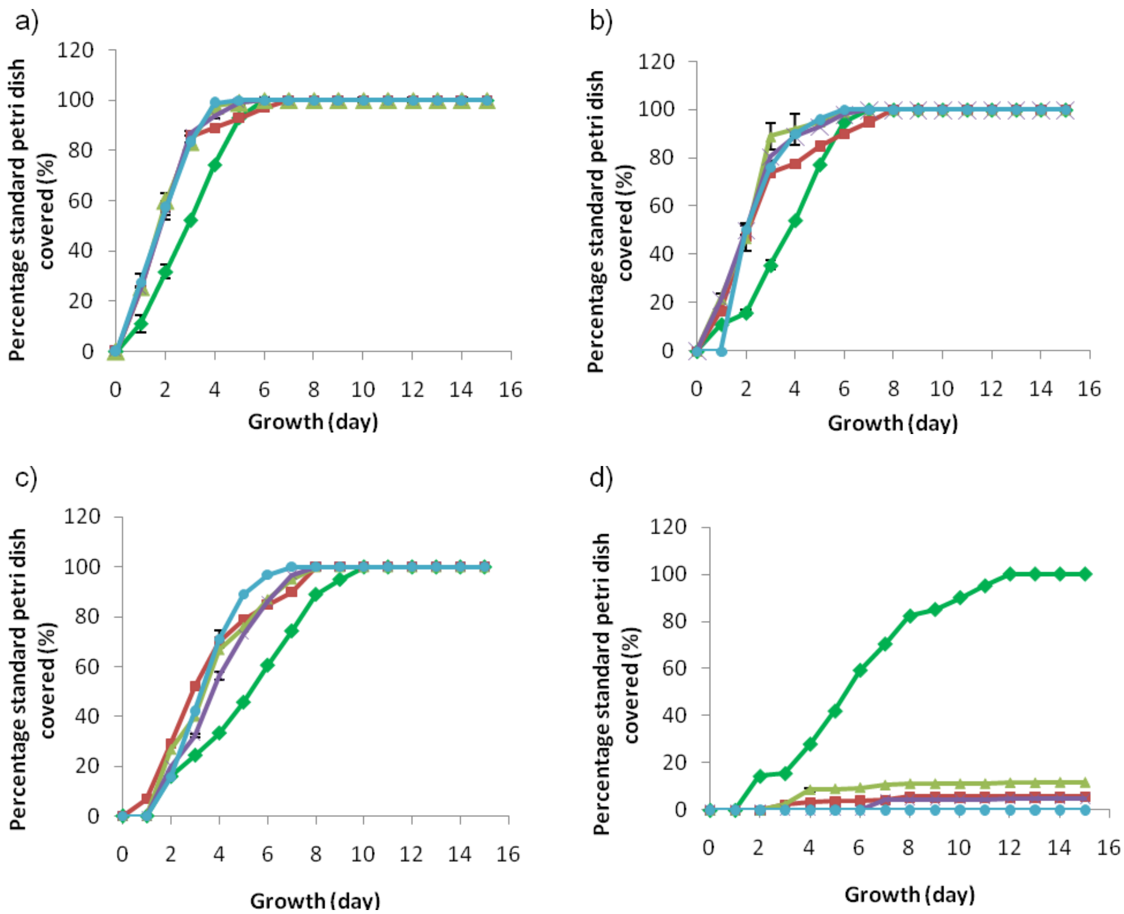

Figure 1. Effect of various concentrations of boric acid on the growth of several species of fungi at 30°C over 15 days in potato dextrose agar: a) 0% (w/v) (control), b) 0.1% (w/v), c) 0.2% (w/v), d) 0.3% (w/v). ♦: *Paecilomyces variotii*, ■: *Trichodema harzanium* I38, ▲: *Trichoderma harzanium* I26, ×: *Trichoderma atroviride*, •: *Trichoderma* sp. Each point represents mean value with the standard deviation of three measurements.

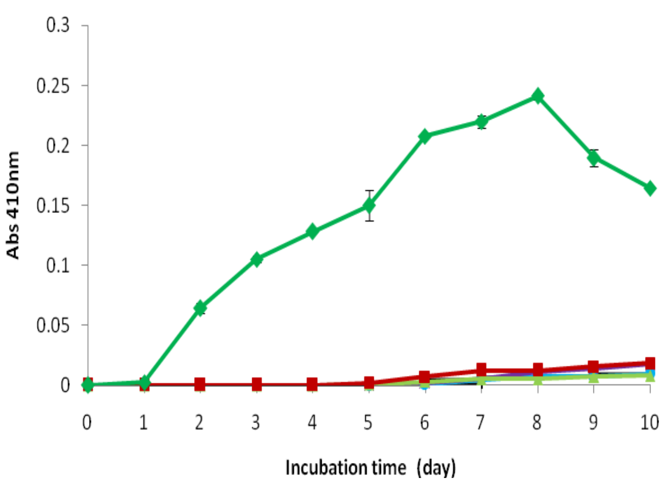

Figure 2. Effect of 0.1% (w/v) boric acid on the production of β-glucosidase in several species of fungi at 37°C over 10 days in liquid medium: ♦: *Paecilomyces variotii*, ■: *Trichodema harzanium* I38, ▲: *Trichoderma harzanium* I26, ×: *Trichoderma atroviride*, •: *Trichoderma* sp. Each point represents mean value with the standard deviation of three measurements.

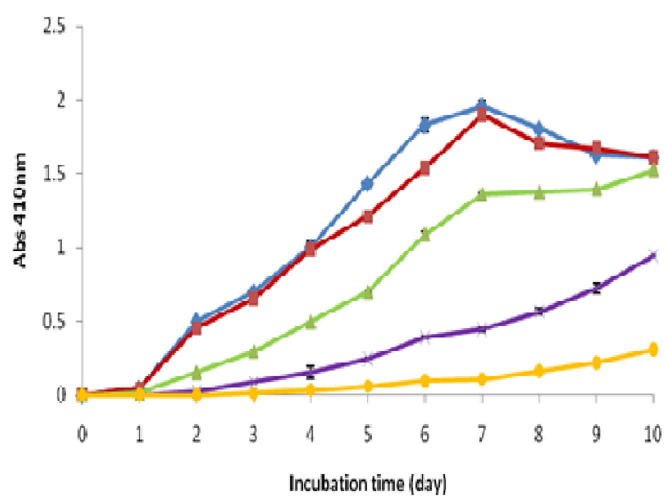

Figure 3. Effect of different concentrations of boric acid on the production of β-glucosidase in *Paecilomyces variotii* at 37°C over 10 days in liquid medium. ♦: 0% (w/v), ■: 0.1% (w/v), ▲: 0.5% (w/v), ×: 1.0% (w/v) •: 1.5% (w/v) boric acid. Each point represents mean value with the standard deviation of three measurements.

REFERENCES

Abbas SQ, Maan A, Iqbal J, Niaz M, Waqas M, Ahmed H, Liaqat A, Sidra (2009). A report of *Paecilomyces variotii* on human from Pakistan. Pak. J. Bot., 41(1): 467-472.

Anita KB, Fernandez V, Rao R (2010). Fungal endophthalmitis caused by *Paecilomyces variotii*, in an immunocompetent patient, following intraocular lens implantation. Indian J. Med. Microbiol., 28(3): 253-254.

Cox C (2004). Boric acid and borates. J. Pest. Reform., 24(2): 10-15.

De Seta F, Schmidt M, Vu B, Essmann M, Larsen B (2009). Antifungal mechanisms supporting boric acid therapy of *Candida vaginitis*. J. Antimicrob. Chemother., 63(2): 325-336.

Hamilton RA, Wolf BC (2007). Accidental boric acid poisoning following the ingestion of household pesticide. J. Forensic Sci., 52(3): 706-708.

Houbraken J, Verweij PE, Rijs AJMM, Borman AM, Samson RA (2010).Identification of *Paecilomyces variotii* in Clinical Samples and Settings. J. Clin. Microbiol., 48(8): 2754-2761.

Lueck E (1980). Antimicrobial food additives: characteristics, uses, effects. Springer-Verlag, New York, pp. 73-77.

Parker BJ, Veness RG, Evans CS (1999). A biochemical mechanism whereby *Paecilomyces variotii* can overcome the toxicity of the wood protectant borate. Enzyme Microb. Technol., 24(7): 402-406.

Salle V, Lecuyer E, Chouaki T, Lescure FX, Smail A, Vaidie A, Dayen C, Schmit JL, Ducroix JP, Douadi Y (2005). *Paecilomyces variotii* fungemia in a patient with multiple myeloma: case report and literature review. J. Infect., 51(3): e93-e95.

See AS, Salleh AB, Bakar FA, Yusof NA, Abdulamir AS, Heng LY (2010). Risk and health effect of boric acid. Am. J. Appl. Sci., 7(5): 620-627.

Strong PL, Robert IK, William CK (2001). Boric Acid and Inorganic Borate Pesticides: Handbook of Pesticide Toxicology (Second Edition). Academic Press, San Diego, pp. 1429-1437.

Tarkkanen A, Raivio V, Anttila VJ, Tommila P, Ralli R, Merenmies L, Immonen I (2004). Fungal endophthalmitis caused by *Paecilomyces variotii* following cataract surgery: a presumed operating room air-conditioning system contamination. Blackwell Science Ltd/Inc, pp. 232-235.