Biocatalysis and Agricultural Biotechnology 3 (2014) 310–315

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Original Research Paper

Indigenous actinomycetes from empty fruit bunch compost of oil palm: Evaluation on enzymatic and antagonistic properties

Adeline Su Yien Ting [a,*], Alex Hermanto [a], Ka Lin Peh [b]

[a] School of Science, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, 46150 Petaling Jaya, Selangor, Darul Ehsan, Malaysia
[b] Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 7 November 2013
Received in revised form
23 February 2014
Accepted 8 March 2014
Available online 25 March 2014

Keywords:
Actinomycetes
Antagonistic activity
EFB compost
Enzymatic activities
Ganoderma control

ABSTRACT

Three actinomycetes isolates were isolated from empty fruit bunch (EFB) compost of oil palm. They were identified as *Nocardiopsis sp.* (ac9), *Streptomyces violaceorubidus* (6ca11) and *Streptomyces sp.* (ac19). The enzymatic potential of the three isolates were established from assays on hydrolytic and lignolytic activities. The production of amylases, cellulases and xylanases were higher in isolates *Nocardiopsis sp.* (ac9) and *S. violaceorubidus* (6ca11), at both mesophilic (40 ± 2 °C) and thermophilic (60 ± 2 °C) temperatures. On the contrary, lignolytic activities were higher at mesophilic temperature. *Streptomyces sp.* (ac19) showed higher lignolytic activities at both temperatures compared to the other two isolates. These three isolates were also antagonistic towards the pathogenic *Ganoderma boninense. Nocardiopsis sp.* (ac9) was superior with 91.4% inhibition. From this study, it is established that indigenous actinomycetes isolates have both enzymatic and antagonistic potential for use in composting and *Ganoderma* control, respectively. We recommend the application of actinomycetes into the compost heap during mesophilic stages to allow progressive composting and for compost to be enriched with actinomycetes.

1. Introduction

Empty fruit bunches (EFB) of oil palm is a major waste problem in oil palm plantations. In the recent years, this waste is managed in a more environmental-friendly approach by converting them into compost. Composting presents an attractive option in EFB management as composting can successfully reduce the initial volume and weight of the EFB by 85% and 50%, respectively (Saletes et al., 2004). Composted EFB also has increased *C:N* ratio and higher nutrient values which benefits crops when the compost is returned to the soil (Lim and Chan, 1987).

The composting process is primarily a microbiological process, regulated by the indigenous microbes found in the compost heap. During composting, organic material is converted to carbon dioxide, humus and heat, by compost microorganisms (Tuomela et al., 2000). As composting takes place, a gradual succession of microbial communities occurs as a response to the different stages of the composting process, namely the mesophilic, thermophilic and curing stage. Various groups of microbes are involved in the composting process, with bacteria more often dominating the mesophilic and thermophilic stages, while fungi and actinobacteria in the curing stage (Tang et al., 2004). Mesophiles predominantly hydrolyse simple sugars and starch, while thermophiles are involved in the breakdown of hemicellulose, cellulose, lignocellulose and lipids. However, complex organic compounds like lignin are mainly degraded by thermophilic fungi and actinomycetes at optimum temperature of 40–50 °C (Tuomela et al., 2000). The expression of the enzymes by the respective groups of microbes is therefore temperature-dependent, and would subsequently impact the rate of composting of the EFB. Efficient composting is highly desirable as it ultimately results in the degradation of 44%, 34% and 20% of cellulose, hemicellulose and lignin residues in EFB, respectively (Saletes et al., 2004).

Indigenous actinomycetes from EFB compost are good candidates for exploration. Actinomycetes are natural antibiotic-producers, able to inhibit various soil-borne pathogens such as *Rhizoctonia solani* (Tuitert et al., 1998; Postma et al., 2003; Patil et al., 2010), *Fusarium oxysporum* f.sp. *cubense* (Getha and Vikineswary, 2002), and *Verticillium dahlia* (Aouar et al., 2012; Cuesta et al., 2012). The role of actinomycetes in composting is favoured by their natural hyphal-branching growth, which allows for the penetration and degradation of insoluble substrates such as lignocellulose (McCarthy, 1987). Therefore, aside from forming an integral part of the microflora responsible for nutrient recycling, actinomycetes contribute immensely in the degradation of lignocellulose substrates in the compost. Another advantage in using actinomycetes for composting is that as prokaryotes, actinomycetes are more amenable to and have higher success rate in strain improvement strategies by genetic manipulation and large-scale

* Corresponding author. Tel.: +603 5514 6105; fax: +603 5514 6364.
E-mail addresses: adelsuyien@yahoo.com, adeline.ting@monash.edu (A.S.Y. Ting).

http://dx.doi.org/10.1016/j.bcab.2014.03.004
1878-8181/

cultivation (McCarthy, 1987). Hence, biomass generation of actinomycetes for compost application is achievable.

In this study, indigenous species of actinomycetes were isolated from EFB compost of oil palm and screened for their hydrolytic and lignolytic activities as well as antagonistic properties towards *Ganoderma boninense*, a serious pathogen of the oil palm. Isolates with these potentials can be useful and further developed for compost application and biological control of *G. boninense*. Both the enzymatic and antagonistic attributes of actinomycetes can hasten the degradation of EFB compost as well as becoming a suitable carrier of biocontrol agents towards *G. boninense* when the compost is applied to the soil.

2. Materials and methods

2.1. Culture establishment

Three isolates of actinomycetes (6ca11, Ac9 and Ac19) were isolated from the EFB compost (curing-stage) obtained from an oil palm mill in Lahad Datu, Sabah, Malaysia. Briefly, isolation was performed using the spread-plate technique, from which pure cultures were subsequently established using the streak-plate technique on Actinomycetes Isolation Agar (AIA) (Difco). The cultures were incubated at 40 ± 2 °C until use.

2.2. Enzymatic analysis

For enzymatic analysis, the cultures were first established by inoculating a loopful of the pure cultures in 30 ml Actinomyces Broth (Difco) and incubated at 40 ± 2 °C and 60 ± 2 °C for 5 days. The cultures were then centrifuged (4600 rpm, 20 min) (Hettich Universal 320R) and the supernatant obtained was filtered through a 0.22 μm membrane pore filter (Sartorius). The cell-free filtrates containing the crude enzymes were then used to assess for hydrolytic (amylase, cellulase, xylanase) and lignolytic activities, using the Dinitrosalicylic acid (DNS) (Wood et al., 2012) and Remazol Brilliant Blue R (RBBR) (Machado et al., 2005) assays, respectively. Triplicates were prepared for each parameter analysed.

Briefly, the DNS assay was conducted using 1.0 ml of crude enzyme extract and 1.0 ml of corresponding substrate in 0.05 M citrate buffer solution (pH 4.5) (R&M Chemicals). For amylase and xylanase assay, the substrate used was 1% (w v^{-1}) soluble starch (Fisher Scientific) and 1% beechwood xylan (Sigma), respectively; while 1.25% (w v^{-1}) of carboxymethylcellulose (CMC) (R&M Chemicals) was used to determine cellulase activities. Control was prepared by substituting enzyme extract with plain actinomyces broth (Difco) at same volume. The mixtures were incubated for 10 min at two different temperatures: 40 ± 2 °C and 60 ± 2 °C. Then, 2 ml of 1% DNS solution (10 g l^{-1} dinitrosalicylic acid (Fluka), 16 g l^{-1} NaOH (Merck), 1 g l^{-1} phenol (R&M Chemicals), 30 g l^{-1} Rochelle salt (sodium potassium tartrate) (R&M Chemicals), 1 g l^{-1} sodium sulphite (J. Kollin Chemicals)) (Wood et al., 2012; Gusakov et al., 2011) was added to allow the reducing sugar (glucose or xylose) to react with the DNS. The reaction was stopped by immersing the mixture in boiling water for 10 min. Finally, the absorbance of the mixture was read using a spectrophotometer (Tecan Infinite 200 Pro NanoQuant) at 540 nm. The reducing sugars liberated due to amylase, cellulase and xylanase activities were calculated from the glucose (50, 100, 150, 200, 250 μg ml^{-1} glucose) (for amylase, cellulase) and xylose (20, 40, 60, 80, 100 μg ml^{-1}) (for xylanase) standard curves constructed, and expressed as the amount of reducing sugars liberated (μg) per minute per ml of enzyme extract added (Sulaiman et al., 2011).

Lignolytic activity was assayed by mixing 2.5 ml of the crude enzyme extracts with 50 μl of 2% RBBR (Sigma) and 100 μl of sterile distilled water. Control was prepared using heat-inactivated crude extracts of the same volume (Machado et al., 2005). The reaction mixture was incubated for 1 h at 40 ± 2 °C and 60 ± 2 °C. The absorbance was then read at 585 nm. RBBR decolourization was determined by the difference between the absorbance values of the test sample and control, and expressed as percentage of decolourization (%) of RBBR.

2.3. in vitro screening for antagonistic activity

in vitro screening for antagonistic activities of the actinomycetes against *G. boninense* (provided by Prof. Dr Sariah Meon, Universiti Putra Malaysia) was carried out by dual culture assay. Actinomycetes isolates were first streaked vertically on Nutrient Agar (NA), 3 cm and 6 cm away from the closest and opposite periphery of the petri dish. The plate was incubated at 37 ± 2 °C for 2 days, followed by the inoculation of a mycelial plug of *G. boninense* (5 mm diameter) at 3 cm away from the actinomycetes streak. This now gives a good placement of 3 cm equal distance of the streak and mycelial plug and from both the periphery of the plate. The inoculated plates were incubated at 37 ± 2 °C for 7 days. Procedure was repeated to prepare the control plates, substituting actinomycetes streak with sterile distilled water. Triplicates were prepared. The inhibitory effect of the actinomycetes towards *G. boninense* is measured by calculating the percentage inhibitory of radial growth (PIRG) as follows:

$$\text{PIRG}(\%) = (\text{R1} - \text{R2})/\text{R1} \times 100\%$$

where R1: radial growth of *G. boninense* in control plate and R2: radial growth of *G. boninense* co-inoculated with actinomycetes.

2.4. Identification of isolates

Identification of the three actinomycetes isolates was performed based on the sequencing of their 16S rRNA gene sequence. The genomic DNA of each isolate was first extracted using the GF-1 commercial kit (Vivantis). Polymerase chain reactions (PCR) were then performed using the actinobacteria-specific primers; S-C-Act-0235-a-S-20: 5′-GGCCTATCAGCTTG TTG-3′ and S-C-Act-0878-a-A-19: 5′-CCGTACTCCCCAGGCGG GG-3′ (Zucchi et al., 2011). The amplified 16S rRNA amplicon was sequenced by First BASE Laboratories Pte. Ltd., and the sequences analysed using BLAST software to determine their homologs.

2.5. Statistical analysis

All statistical analysis was analysed using ANOVA performed with the R software (Knezevic et al., 2007). Means were compared with Tukey's tests ($HSD_{(0.05)}$) or a *T*-test ($\alpha = 0.05$) where appropriate.

3. Results

3.1. Hydrolytic and lignolytic activities

Of the three isolates tested, ac9 and 6ca11 produced relatively higher levels of amylase, cellulase and xylanase, compared to ac19 (Fig. 1A and B). Ac9 and 6ca11 have comparable cellulase and xylanase activities at both incubation temperatures; while amylase activity was significantly higher in ac9 with 477.734 μg min^{-1} ml^{-1} against 345.970 μg min^{-1} ml^{-1} by 6ca11 at 40 ± 2 °C (Fig. 1A). Hydrolytic activities of ac19 were inferior to both ac9 and 6ca11 at both temperatures.

Higher enzymatic activities were also observed when isolates were incubated at a higher temperature (60 ± 2 °C) (Fig. 1B),

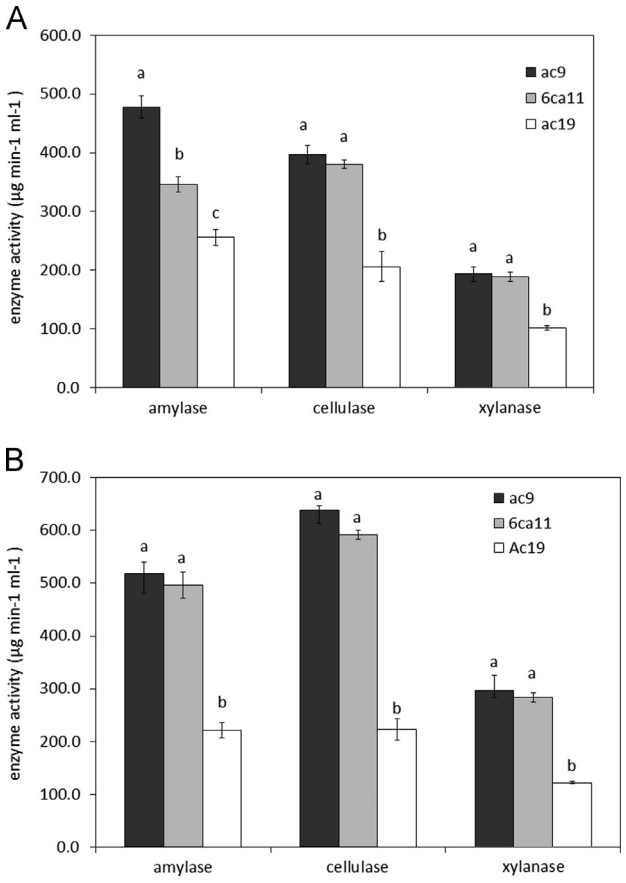

Fig. 1. Enzymatic activities of isolates *Nocardiopsis sp.*(ac9), *Streptomyces violaceorubidus* (6ca11) and *Streptomyces sp.* (ac19) at (A) 40 ± 2 °C and at (B) 60 ± 2 °C. Means with the same letters for the same enzyme are not significantly different (Tukey's test, $\alpha = 0.05$). Bars indicate standard errors.

suggesting possible thermotolerant nature of isolates and the thermostability of the enzymes. Isolate 6ca11 was observed to benefit the most in high temperature condition, showing significant increases in amylase, cellulase and xylanase activities with 496.383, 592.418 and 283.218 $\mu g\ min^{-1}\ ml^{-1}$, compared to 345.970, 380.970 and 188.812 $\mu g\ min^{-1}\ ml^{-1}$, respectively at 40 ± 2 °C (Fig. 2A). Contrary, isolates ac9 and ac19 produced different levels of enzyme activities in response to the higher temperature. Although cellulase and xylanase activities were significantly higher for isolate ac9 at 60 ± 2 °C, amylase activities were not significantly increased (Fig. 2B). For isolate ac19, only xylanase activity was significantly higher at 60 ± 2 °C (Fig. 2C).

Lignolytic activities by ac9, ac19 and 6ca11 were discovered to be temperature sensitive. Lignolytic activities were detected at 40 ± 2 °C for all isolates, and were generally higher compared to activities at 60 ± 2 °C (Fig. 3). At 60 ± 2 °C, ac9, ac19 and 6ca11 recorded only 3.42, 9.92 and 1.33% of RBBR decolorization, respectively (Fig. 3). These values were a significant contrast to the % of decolourization by the isolates at 40 ± 2 °C, which recorded 15.22, 15.78 and 3.67%, respectively (Fig. 3).

3.2. Antagonistic activities

All three isolates showed good antagonistic activities towards *G. boninense* in the dual culture plate assay. Isolates ac9 and 6ca11 were superior compared to isolate ac19, with PIRG values of 91.4% and 86.4%, respectively against 69.1% by ac19 (Fig. 4). Inhibition was primarily due to the inhibitory compounds produced as growth was clearly inhibited at the clearing zone (inhibition zone). Competitive exclusion via overgrowth and mycoparasitism was not observed in all three isolates (data not shown).

3.3. Identification of isolates

Isolate ac9 was identified as *Nocardiopsis sp.* with 99% similarity (602 bp/606 bp) to *Nocardiopsis sp.* (JN942137.1) based on their 602 bp sequence after BLAST analysis. For isolate 6ca11, it was identified as similar to *Streptomyces violaceorubidus* (99% similarity, 527bp/528 bp). The final isolate (ac19) was identified as *Streptomyces sp.* (100% similarity, 619 bp/619 bp).

4. Discussion

This study generated some novel findings especially on the discovery of indigenous actinomycetes from EFB with antagonistic properties towards *G. boninense*, and the preliminary profiling of their enzymatic capacity to enhance the composting process. Two of the actinomycetes isolates were *Streptomyces spp.* (6ca11 and ac19), which are typical species of the actinomycetes community (Miyashita et al., 1982; Cuesta et al., 2012). The third isolate, a *Nocardiopsis*, is a non-*Streptomycete* actinomycetes with distinct lineage within the order of Actinomycetales (Rainey et al., 1996). The discovery of *Nocardiopsis sp.*(ac9), *S. violaceorubidus* (6ca11) and *Streptomyces sp.* (ac19), and their antagonistic potential towards *G. boninense* (particularly *Nocardiopsis sp.* (ac9) (91.4% PIRG)), presents an interesting option to the biocontrol strategies for *G. boninense*. To date, reports on the bioactivity of *Nocardiopsis sp.* (ac9) and *Streptomyces spp.* (6ca11, ac19) towards *G. boninense* is lacking. Instead, *Nocardiopsis sp.* is more established for their antibacterial activities towards a host of human pathogens such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterococcus faecalis* and *Staphylococcus aureus* (Vimal et al., 2009). Antagonism of *Streptomyces spp.* is more common than *Nocardiopsis spp.* (Aouar et al., 2012), with known broad-spectrum antibacterial and antifungal activities towards *Micrococcus luteus, Candida albicans, Salmonella, S. aureus* (Bensultana et al., 2010), and *Sclerotium rolfsii* and *R. solani* (Boukaew et al., 2011). Therefore, this study may be the first few to document that *Nocardiopsis sp.* (ac9), *S. violaceorubidus* (6ca11) and *Streptomyces sp.* (ac19) from EFB compost, have potential as biocontrol agents towards *G. boninense*.

The three isolates from this study can be considered as possible thermophiles based on the definition purposed by Taha et al. (2007). In their study, thermophilic nature is proven when isolates are able to tolerate incubation period at 44 °C for 7 days. In this study, cultures grew very well at 40 ± 2 °C although incubation at 60 ± 2 °C did not produce colony growth on AIA plates (data not shown). As such, we consider our isolates as tolerant to mesophilic and initial thermophilic temperatures, able to withstand the composting process as they were successfully isolated from the curing-staged composts. The thermo-tolerant nature of all three isolates is further strengthen with earlier reports of similar thermophilic characteristics in species of *Streptomyces* (Strom, 1985) and *Nocardiopsis* (Stamford et al., 2001; Yan et al., 2011).

It is apparent that the thermostable enzymes produced by *Nocardiopsis sp.*(ac9), *S. violaceorubidus* (6ca11) and *Streptomyces sp.* (ac19) observed in this study, were most probably attributed to the inherent thermophilic nature of the isolates. Although the actinomycetes isolates were not extreme thermophiles, their extracellular enzymes were thermostable. This was evident when hydrolases have higher activities at 60 ± 2 °C, despite the inability of the isolates to grow at the same temperature on AIA plates. The extracellular enzymes produced by these isolates are significant as they can accelerate substrate degradation through a process that is

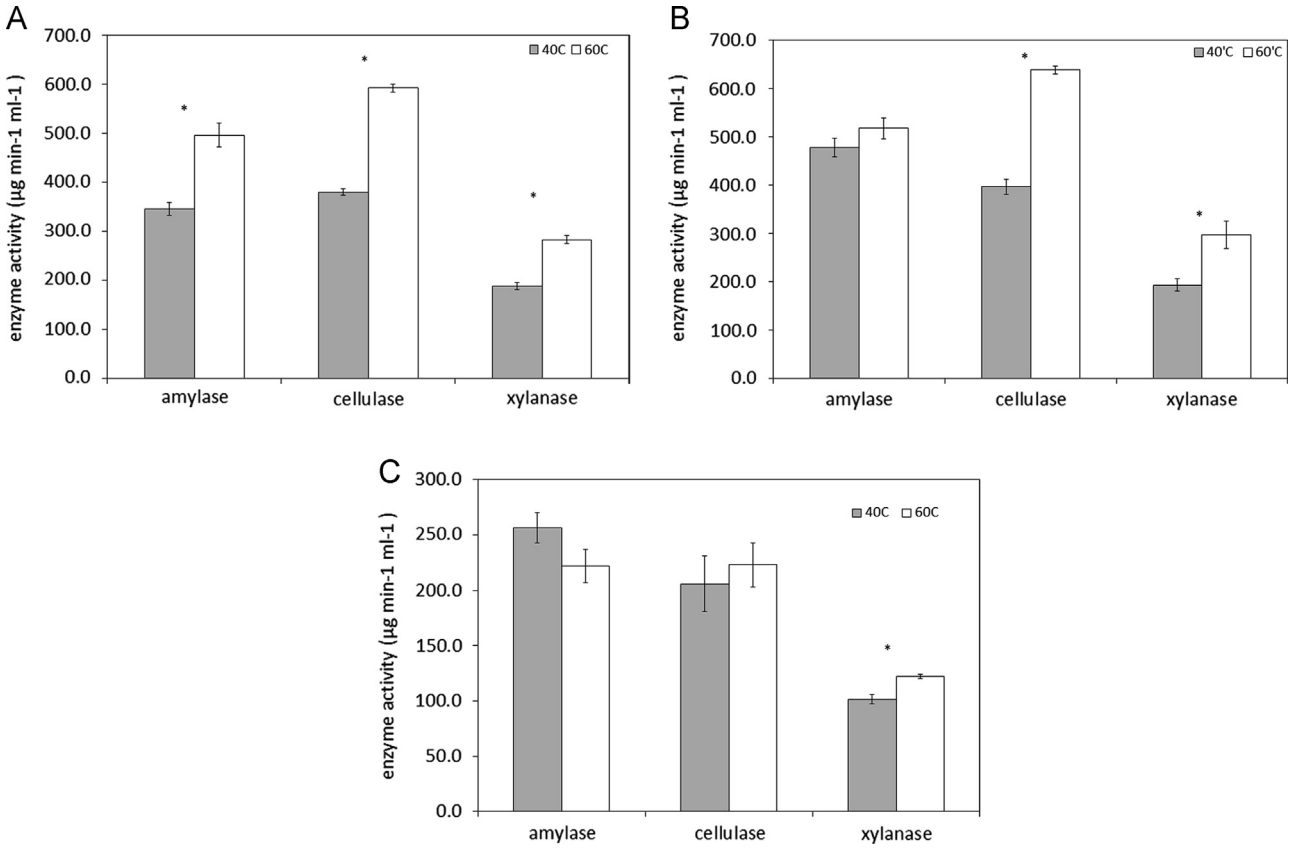

Fig. 2. Comparison of enzymatic activities at 40 ± 2 °C and 60 ± 2 °C for isolates (A) *S. violaceorubidus* (6ca11) (B) *Nocardiopsis sp.*(ac9), and (C) *Streptomyces sp.* (ac19). The "*" symbol indicates significance of *T*-test ($\alpha = 0.05$). Bars indicate standard errors.

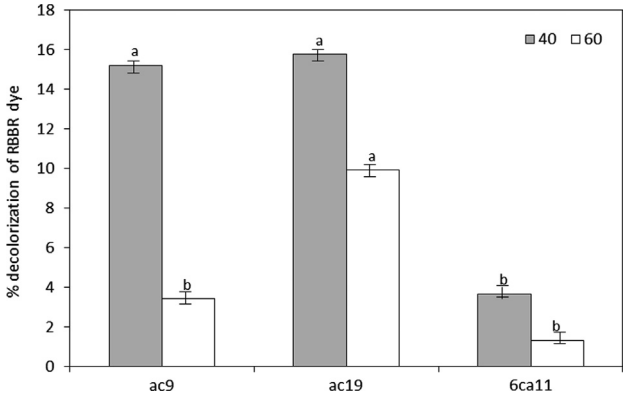

Fig. 3. Lignolytic activities of *Nocardiopsis sp.*(ac9), *S. violaceorubidus* (6ca11) and *Streptomyces sp.* (ac19) at 40 ± 2 °C and 60 ± 2 °C. Means with the same letters for the same temperature are not significantly different (Tukey's test, $\alpha = 0.05$). Bars indicate standard errors.

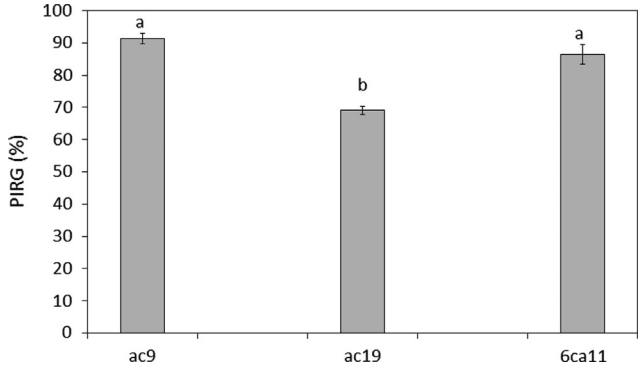

Fig. 4. Percentage of inhibition of radial growth (PIRG, %) by isolates *Nocardiopsis sp.*(ac9), *S. violaceorubidus* (6ca11) and *Streptomyces sp.* (ac19) when co-inoculated with *Ganoderma boninense.* Means with the same letters are not significantly different (Tukey's test, $\alpha = 0.05$). Bars indicate standard errors.

independent of cells. This cell-independent process was also observed in our previous study using the thermophilic bacterium *Ureibacillus terrenus* (Ting et al., 2013). We also observed a distinctive variant in the thermostability of enzymes within species of *Streptomyces* as *S. violaceorubidus* (6ca11) produced more thermostable amylases, cellulases and xylanases, compared to *Streptomyces sp.* (ac19). Our results for *Nocardiopsis sp.* (ac9) also differed from other reports where optimum temperatures for hydrolytic activities varied. We postulate this may be due to the origin of the *Nocardiopsis sp.* and their subsequent response to temperature. In this study, higher cellulase and xylanase activities of *Nocardiopsis sp.* (from compost) were reported at 60 ± 2 °C while amylase activities were not significantly different at both temperatures. This did not resemble observations by Stamford et al. (2001) who identified the optimum amylolytic activity for the endophytic *Nocardiopsis* strain from yam bean was at 70 °C, and at 40 °C for cellulolytic and xylanase activity for a *Nocardiopsis* strain from soil (Saratale and Oh, 2011).

Contrary to observations for hydrolytic activities, all three actinomycetes isolates were found to have better ligninase activities at 40 ± 2 °C than at higher temperature. This suggested the possibility that ligninase-activity is a cell-dependent process, in which when cell growth is affected by the high temperature; the ligninase activities were also affected. Comparisons with ligninase activities of thermophilic *U. terrenus* further reiterate this as thermophilic bacterial growth at 60 ± 2 °C leads to significantly

higher ligninase activities (Ting et al., 2013). Among the three isolates tested, the ligninase activities of *Streptomyces sp.* (ac19) were the highest at both temperatures. This was rather unexpected as hydrolytic enzyme levels for ac19 were inferior to the other two isolates. Therefore, it appears that for *Streptomyces sp.* (ac19), ligninases and not cellulases could be primarily involved in the breakdown of lignocellulose compounds. Mason et al. (1988) postulated this as well when the molecular weight of a single enzyme by *Streptomyces cyaneus* on ball-milled straw, capable of solubilizing lignin in lignocellulose, was identified as neither a cellulase nor a xylanase. Hence, it would appear as all three actinomycetes isolates in this study can produce both hydrolytic and lignolytic activities, which are useful for degradation of lignocellulose compounds at low and high temperatures, respectively. The identification of the specific enzymes produced by the various actinomycetes was however not further investigated in this study.

Although this study has shown the hydrolytic and lignolytic potential of actinomycetes, their enzymatic activities were relatively lower compared to activities from soil bacteria such as *Bacillus spp*. (Kirby, 2005; Mayende et al., 2006; Guo et al., 2012; Hashemi et al., 2012). The inferiority of enzyme activities of actinomycetes in this study compared to other bacterial isolates may indicate that actinobacteria by nature produces less enzymes than bacteria. We also did not rule out the possibility that our lower levels were due to the use of non-purified enzyme extracts. Purification is highly recommended to remove reducing agents present in crude extracts (Heck et al., 2005; Guo et al., 2012).

In view of their response to temperature, antagonistic actinomycetes are recommended for incorporation into the compost at early composting stages to allow high population densities to develop into the curing stage (Tuitert et al., 1998). This strategy is feasible to generate EFB compost enhanced with antagonistic actinomycetes against *G. boninense*. Although there are no reported attempts in using compost enriched with actinomycetes for *Ganoderma* control, this approach has been demonstrated to be successful in suppressing disease in other crops. This includes control of *R. solani* by cellulolytic and oligotrophic actinomycetes inhabiting long-matured compost (Tuitert et al., 1998). Addition of compost enriched with actinomycetes to soils has also been shown to increase the incidence of beneficial rhizobacteria (Escuadra and Amemiya, 2008) which in turn, has antagonistic activities against a variety of soil-borne pathogens such as *F. oxysporum* f.sp. *radicis-lycopersici, Pythium ultimum* and *R. solani* (deBrito et al., 1995). As such, we expect the incorporation of *Nocardiopsis sp.*(ac9), *S. violaceorubidus* (6ca11) and *Streptomyces sp.* (ac19) into EFB compost to render similar beneficial effects against *G. boninense.*

To conclude, this study has successfully identified three indigenous actinomycetes isolates from EFB compost, and documented their enzymatic and antagonistic potential. This preliminary observation allows the further investigations and development into harnessing the biomass or the enzyme derivatives of *Nocardiopsis sp.*(ac9), *S. violaceorubidus* (6ca11) and *Streptomyces sp.* (ac19), to accelerate the composting process and for control of *Ganoderma.*

Acknowledgements

This work was partly supported by the Fundamental Research Grant Scheme (FRGS) (FRGS/2/2010/SG/MUSM/03/2) from the Malaysian Ministry of Higher Education. The authors also extend their gratitude to Monash University Malaysia for the facilities to complete the project.

References

Aouar, L., Lerat, S., Ouffroukh, A., Boulahrouf, A., Beaulieu, C., 2012. Taxonomic identification of rhizospheric actinobacteria isolated from Algerian semi-arid soil exhibiting antagonistic activities against plant fungal pathogens. Can. J. Plant Pathol. 34 (2), 165–176.

Bensultana, A., Ouhdouch, Y., Hassani, L., Mezrioui, N., Rafouk, L., 2010. Isolation and charcterization of wastewater sand filter actinomycetes. World J. Microbiol. Biotechnol. 26, 481–487.

Boukaew, S., Chuenchit, S., Petracharat, V., 2011. Evaluation of *Streptomyces* spp. for biological control of *Sclerotium* root and stem rot and *Ralstonia* wilt of chili pepper. Biocontrol 56 (3), 365–374.

Cuesta, G., Garcia-de-la-Fuente, R., Abad, M., Fornes, F., 2012. Isolation and identification of actinomycetes from a compost-amended soil with potential as biocontrol agents. J. Environ. Manage. 95, S280–S284.

deBrito, A.M., Gagne, S., Antoun, H., 1995. Effect of compost on rhizosphere microflora of the tomato and on the incidence of plant growth-promoting rhizobacteria. Appl. Environ. Microbiol. 61 (1), 194–199.

Escuadra, G.M.E., Amemiya, Y., 2008. Suppression of Fusarium wilt of spinach with compost amendments. J. Gen. Plant Pathol. 74 (4), 267–274.

Getha, K., Vikineswary, S., 2002. Antagonistic effects of *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum* f.sp. *cubense* race 4: indirect evidence for the role of antibiosis in the antagonistic process. J. Indus. Microbiol. Biotechnol 28 (6), 303–310.

Guo, G., Liu, Z., Xu, J., Liu, J., Dai, X., Xie, D., Peng, K., Feng, X., Duan, S., Zheng, K., Cheng, L., Fu, Y., 2012. Purification and characterization of a xylanase from *Bacillus subtilis* isolated from the degumming line. J. Basic Microbiol. 52, 419–428.

Gusakov, A.V., Kondratyeva, E.G., Sinitsyn, A.P., 2011. Comparison of two methods for assaying reducing sugars in the determination of carbohydrase activities. Int. J. Analy. Chem. 2011, 1–4.

Hashemi, M., Mousavi, S.S., Razavi, S.H., Shojaosadati, S.A., 2012. Comparison of submerged and solid state fermentation systems effects on the catalytic activity of *Bacillus sp*. KR-8104 α-amylase at different pH and temperatures. Ind. Crops Products. 43, 661–667.

Heck, J.X., Flores, S.H., Hertz, P.F., Ayub, M.A.Z., 2005. Optimization of cellulase-free xylanase activity produced by *Bacillus coagulans* BL69 in solid-state cultivation. Process Biochem. 40, 107–112.

Kirby, R., 2005. Actinomycetes and lignin degradation. Adv. Appl. Microbiol. 58, 125–168.

Knezevic, S.Z., Streibig, J.C., Ritz, C., 2007. Utilizing R software package for dose-response studies: the concept and data analysis. Weed Technol. 21 (3), 840–848.

Lim, K.H., Chan, K.W., 1987. Towards optimising empty fruit bunch application in oil palm. In: Proceedings of the International Oil Palm/Palm Oil Conference. MPOB, Kuala Lumpur.

Machado, K.M.G., Matheus, D.R., Bononi, V.L.R., 2005. Ligninolytic enzymes production and remazol brilliant blue R decolorization by tropical Brazilian basidiomycetes fungi. Braz. J. Microbiol 36 (3), 246–252.

Mason, J.C., Richards, M., Zimmermann, W., Broda, P., 1988. Identification of extracellular proteins from actinomycetes responsible for the solubilisation of lignocellulose. Appl. Microbiol. Biotechnol. 28 (3), 276–280.

Mayende, L., Wilhelmi, B.S., Pletschke, B.I., 2006. Cellulases (CMCases) and polyphenol oxidases from thermophilic *Bacillus spp*. isolated from compost. Soil Biol. Biochem. 38, 2963–2966.

McCarthy, A.J., 1987. Lignocellulose-degrading actinomycetes. FEMS Microbiol. Lett. 46 (2), 145–163.

Miyashita, K., Kato, T., Tsuru, S., 1982. Actinomycetes occurring in soil applied with compost. Soil Sci. Plant Nutr. 28 (3), 303–313.

Patil, H.J., Srivastava, A.K., Kumar, S., Chaudhari, B.L., Arora, D.K., 2010. Selective isolation, evaluation and characterization of antagonistic actinomycetes against *Rhizoctonia solani*. World J. Microbiol. Biotechnol. 26 (12), 2163–2170.

Postma, J., Montanari, M., van de Boogert, P.H.J.F., 2003. Microbial enrichment to enhance the disease suppressive activity of compost. Eur. J. Soil Biol. 39 (3), 157–163.

Rainey, F.A., Ward-Rainey, N., Kroppenstedt, R.M., Stackebrandt, E., 1996. The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. Int. J. Evol. Sys. Microbiol. 46 (4), 1088–1092.

Saletes, S., Siregar, A.F., Caliman, J.P., Liwang, T., 2004. Ligno-cellulose composting: case study on monitoring oil palm residuals. Compost. Sci. Util. 12 (4), 372–382.

Saratale, G.D., Oh, S.E., 2011. Production of thermotolerant and alkalotolerant cellulolytic enzymes by isolated *Nocardiopsis* sp. KNU. Biodegradation 22 (5), 905–919.

Stamford, T.L.M., Stamford, N.P., Coelho, L.C.B.B., Araujo, J.M., 2001. Production and characterization of a thermostable α-amylase from *Nocardiopsis* sp. endophyte of yam bean. Biores. Technol. 76 (2), 137–141.

Strom, P.F., 1985. Identification of thermophilic bacteria in solid-waste composting. Appl. Environ. Microbiol. 50 (4), 906–913.

Sulaiman, F., Abdullah, N., Gerhauser, H., Shariff, A., 2011. An outlook of Malaysian energy, oil palm industry and its utilisation of wastes as useful resources. Biomass Bioenergy 35, 2011.

Taha, M.P.M., Drew, G.H., Vestlund, A.T., Aldred, A., Longhurst, P.J., Pollard, S.J.T., 2007. Enumerating actinomycetes in compost bioaerosols at source—use of soil compost agar to address plate 'masking'. Atmos. Environ 41 (22), 4759–4765.

Tang, J.C., Kanamori, T., Inoue, Y., Yasuta, T., Yoshida, S., Katayama, A., 2004. Changes in the microbial community structure during thermophilic composting of manure as detected by the quinone profile method. Process Biochem. 39 (12), 1999–2006.

Ting, A.S.Y., Tay, H.Q., Peh, K.L., Tan, W.S., Tee, C.S., 2013. Novel isolation of thermophilic *Ureibacillus terrenus* from compost of empty fruit bunches (EFB) of oil palm and its enzymatic activities. Biocatal. Agric. Biotechnol. 2, 162–164.

Tuitert, G., Szczech, M., Bollen, G.J., 1998. Suppression of *Rhizoctonia solani* in potting mixtures amended with compost made from organic household waste. Phytopathology 88, 764–773.

Tuomela, M., Vikman, M., Hatakka, A., Itavaara, M., 2000. Biodegradation of lignin in a compost environment: a review. Biores. Technol. 72 (2), 169–183.

Vimal, V., Rajan, B.M., Kannabiran, K., 2009. Antimicrobial activity of marine actinomycete, *Nocardiopsis* sp. VITSVK 5 (FJ973467). Asian J. Med. Sci. 1, 57–63.

Wood, I.P., Elliston, A., Ryden, P., Bancroft, I., Roberts, I.N., Waldron, K.W., 2012. Rapid quantification of reducing sugars in biomass hydrolysates: improving the speed and precision of the dinitrosalicylic assay. Biomass Bioenergy 44, 117–121.

Yan, X., Yan, H., Liu, Z., Liu, X.D., Mo, H.P., Zhang, L.P., 2011. *Nocardiopsis yanglingensis* sp. nov., a thermophilic strain isolated from a compost of button mushrooms. Anton. Leeuwen 100 (3), 415–419.

Zucchi, T.D., Guidolin, A.S, Consoli, F.L., 2011. Isolation and characterization of actinobacteria ectosymbionts from *Acromyrmex subterraneus brunneus*. Microbiol. Res. 166, 68–76.