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Antagonistic Activity of Endophytic *Bacillus* Species Against *Colletotrichum Gloeosporioides* for the Control of Anthracnose Disease in Black Pepper (*Piper Nigrum L.)*

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Abstract

Anthracnose caused by *Collectotrichum gloeosporioides* is a serious disease of black pepper. The antagonistic effect of three *Bacillus species*, i.e. *Bacillus strain CBF, YCA0098 and YCA5593,* were tested against *Collectotrichum gloeosporioides in vitro* and *in vivo*. *In vitro* test showed that all *Bacillus* species significantly reduce the mycelia growth and spore germination of the C. *gloeosporioides.* Scanning electron microscopy revealed significant inhibition of *C. gloeosporioides* spore germination on pepper leaves surface. Combination of bacterial strain CBF, YCA0098 and YCA5593 maintained but not increased the inhibitory effect on spore germination of *C. gloeosporioides* when cells were co-incubated with *C. gloeosporioides* and when the pathogen was incubated in mixture of cell-free culture extracts. Combination of strains maintained efficacy in control of *C. gloeosporioides* in pepper vines compared to individual strains, but reduced variability and improved consistency between experiments, especially mixture of strain CBF, YCA0098 and YCA5593.

Keywords: Anthracnose, Antagonistic, Black pepper, *Bacillus, Collectotrichum*

1.0 INTRODUCTION

Black pepper (*Piper nigrum L.)* is one of the important export commodities in Malaysia, with production area of approximately 16,021 hectare (MPIC, 2013). Leaves anthracnose disease (also known as black berries disease), caused by *Collectotrichum gloeosporioides*, is a worldwide disease of black pepper and caused up to 50% yield losses and reduction of berries quality (Wong, 2002). *Collectotrichum gloeosporioides* overwinter as conidia in plant debris in the soil and generally infects plants as mycelia originating from these conidia or airborne ascospores that directly penetrate host leaves or berries tissue (Agrios, 2005).

Several approaches have been used to prevent, mitigate or control plant diseases. Currently, although the uses of chemical are comparatively suitable to control the fungal diseases, but continuous and abusive application has lead to the apparition of environment and human health problems. Also, traditional pepper breeding program for disease resistance has been hampered by limited gene pool (Lau et al., 2013) and inconsistent results produced (Chen, 2014).

Biological control using antagonistic bacteria has been considered as an alternative disease management strategy due to its potential to provide safe and environmentally compatible disease control (El-Kot, 2008). However, the major problem of biological control is the lack of consistency due to variable efficacy of the biological control agent dependent on the soil environment where the biocontrol agent is applied, the moment and the method of application, the host plant or the pathogen species. In addition, appropriate formulations would also be another challenges for successful implementation of biological control. Published data on this area remain sparse perhaps because they may involve industry secret in comparison with the considerable volume of literature describing selection procedure, mechanism of action or genetics of biological control agents. One approach to overcome inconsistent performance by biological control agent is through integration of multiple microbes into individual biological control formulations (Asghar and Pessarakli, 2010), and a second approach is through diversified the application method (Zhang et al., 2010). With regards to *Collectotrichum gloeosporioides on* black pepper, application of microbial biological control agent may improve disease suppression as these pathogens can infect the host near the soil line and in the foliar canopy.

Species belonging to *Bacillus* is frequently used as biocontrol agents, since they excrete hydrolytic enzymes that are able to degrade cell walls (Chernin and Chet, 2002), iron-chelating siderophores, several cyclic lipodepsipeptides (LDP) (Dalla *et al*., 2003), as well as a great variety of antibiotics such as, iturin (Joshi and Gardner, 2006; Tsuge *et al*., 2001; Phister *et al*., 2004), surfactin (Peypoux *et al*., 1999; Ajlani *et al*., 2007 and Huszcza & Burczyk, 2006), fengycin, (Loeffler *et al*., 1986 and Liu *et al*., 2011), bacillomycin (Athukora et al., 2009, Liu et al., 2011 and Ramarathnam *et al*., 2007) and mycosubtilin (Leclere *et al*., 2005). The objective of the present study were to determine the efficiency and efficacy of *Bacillus strains* CBF, YCA0098 and YCA5593 to control anthracnose disease in black pepper under both greenhouse and field experiments.

2.0 MATERIAL AND METHODS

2.1 Bacteria, fungi and plant materials

The bacterial identifications and the origins of the 3 strains of *Bacillus* spp used in this study are presented in Table 1. Pure cultures of each bacterial cells were maintained in Luria-Bertani broth, amended with 20% glycerol (Fisher Scientific) and stored at -80^{o}C. The bacterial cells were routinely cultured in Luria-Bertani (LB) broth supplemented with