## BO2 - Biodiversity Inventory, Collection and Preservation (in-situ and ex-situ):Prokaryotes and Leaf Blight Pathogenic Fungi on Oil Palm

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#### Abstract

Chitinase and  $\beta$ -glucanase have a role as a biological control agent of oil palm pathogenic fungi, such as *Curvularia affinis* and *Colletotrichum gloeosporioides*. The pathogens caused antrachnose, leaf blight, and rotting on oil palm leaves. There are two bacterial isolates selected as chitinase and  $\beta$ -glucanase producer which isolated from soil of oil palm plantation in Jambi. *Bacillus thuringiensis* SAHA 12.08 produced optimum chitinase at 60-h incubation. The chitinase was precipitated by 30% ammonium sulphate and the activity increased 2.35 fold. *Bacillus subtilis* SAHA 32.6 produced optimum  $\beta$ -glucanase at 12-h incubation. The  $\beta$ -glucanase was precipitated by 60% ammonium sulphate and could increase 1.43 fold its activity. Both precipitated chitinase of *B. thuringiensis* SAHA 12.08 and  $\beta$ -glucanase of *B. subtilis* SAHA 32.6 could inhibit the growth of *C. affinis* and *C. gloeosporioides* by using in vitro test. The isolates and also the enzymes showed potential to apply as biocontrol agents for fungal pathogens on oil palm plants.



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#### **Growth Curve and Enzyme Activity of Bacterial Isolates**

Fig. 1 Growth curve and enzyme activity of (A) *Bacillus thuringiensis* SAHA 12.08 and (B) *Bacillus subtilis* SAHA 32.6



Table 1 Chitinase partially purification of *B. thuringiensis* SAHA 12.08

Steps	Total Protein (mg)	Total Activity (U)	Specific (U <sup>/</sup> mg protein)	Purification (fold)
Crude extract	19.68	142.6	7.24	1
30% ammonium sulphate precipitation	0.0842	1.44	17.061	2.35



# Effectiveness of *B. thuringiensis* SAHA 12.08 against Leaf Blight Disease (Detached Leaf Assay)

Crude enzyme has inhibitory against *C. affinis* better than 60h cell culture. *C. gloeosporioides* 60-h cell culture capable of inhibiting better than the crude enzyme. This chitinase could inhibit *C. affinis* than *C. gloeosporioides* through in vitro and detached leaf assay and has potential application as biocontrol agents for *C. affinis* and *C. gloeosporioides*. Fig. 4 Effectiveness of *B. thuringiensis* SAHA 12.08 against leafblight disease using oil palm leaves

Table 2 β-glucanase partially purification of *B. subtilis* SAHA 32.6

Steps	Total Protein (mg)	Total Activity (U)	Specific Act. (U/mg protein)	Purification (fold)
Crude extract	12.48	4.48	0.36	1
60% ammonium sulphate precipitation	0.114	0.06	0.51	1.31

### Antagonistic Activity

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Fig. 2 Leaf blight disease on oil palm leaves (A) caused by *Curvularia affinis* and *Colletotrichum gloeosporioides* (B)







Fig. 5 Leaf structure of oil palm after treatment with (a) *C. affinis*, (b) *C. affinis* + *B. thuringiensis*, (c) negative control (without treatment): 1) upper epidermis, 2) bundle sheath,
3) mesophyll, 4) lower epidermis



Fig. 3 The antagonistic activity of chitinolytic *B. thuringiensis* SAHA 12.08 against *C. affinis* (A) and *C. gloeosporioides* (B) after 7 days incubation on PDA medium. Aquadest used as negative control. Precipitated enzyme by using 30% ammonium sulphate.

The antagonistic activity of glucanolytic *B. subtilis* SAHA 32.6 against *C. affinis* (C) and *C. gloeosporioides* (D) after 7 days incubation on PDA medium.

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Fig. 6 Leaf structure of oil palm after treatment with (a) *C. gloeosporioides*, (b) *C. gloeosporioides* + *B. thuringiensis*, (c) negative control (without treatment): 1) epidermis, 2) mesophyll, 3) bundle sheath

