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nzymatic Characterization of Three Entomopathogenic Fungi with the API ZYM System【Scientific note】

以API ZYM系統測定蟲生真菌之酵素特性【科學短訊】

Wen-Feng Hsiao
蕭文鳳

*通訊作者E-mail :

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Abstract

The enzymatic profiles of Beauveria bassiana (Bb), Metarihizium anisopliae (Ma), Verticillium lecanii (VI), and their subcultures were studied by using the API ZYM system. Isolates all showed no reaction to substrates with β-glucuronidase, or cystein aminopeptidase except for Ma (str. 195). All isolates showed little sctivity or were completely without activity to substrates with lipase, trysin, chymotrysin, α-glucosidase, orα-fucosidase. However, Bb Ho 42 reacted strongly to substrate with α-mannosidase. Both isolates of M. anisopliae (strs. 194 and 195) reacted strongly to substrates with leucine aminopeptidase, acid phosphatase, phosphoamidase, orβ-glucosaminidase. Additionally, M. anisopliae str. 194 reacted strongly to the substrates with esterase (C4), or esterase lipase, while M. anisoplaie str. 195 moderately reacted to the substrates with β-glucosidase. Results showed that the enzymatic activity of B. bassiana against substrates with phosphatase alkaline, leucine aminopeptidase, α-galactodase, orα-galactodase declined after serial passages from subcultures of Ho. 212 F3 to F9. V. lecanii exhibited the tendency of declining activity against substrates with leucine aminopeptidase, valine aminopeptidase, phosphosamidase, β-glucosidase, orβ-glucosaminidase from subcultures of F4 to F6. On the other hand, the reaction to the substrate with phosphatase alkline increased. The results of this study indicate that API ZYM techniques provide a rapid and reproducible semi-quantitative method for revealing enzymatic activity.

摘要

本實驗利用半定量之API ZYM 系統來研究三種蟲生真菌，白殭菌Beauveria bassiana ，黑殭菌 Metarhizium anisopliae，蠟蚧幹枝孢菌Verticillium lecanii的酵素剖圖。所有的分離株對β-glucuronidase 及cysteine aminopeptidase 的基質無反應，但黑殭菌195菌株對後者有反應。所有的分離株對Lipase, trypsin, chymotrypsin, α-glucos idase 及α-fucosidase 的基質無反應或只有輕微的反應。而白殭菌 Col 42品系對α-mann osidase基質有很強的反應。 黑殭菌的二個分離株(194及195)對leucine aminopeptidase, phosphatase acid, phosphoamidase, β-glucosaminidase具有強烈之反應。除此外，黑殭菌194對esterase, esterase lipase和β-glucosidase 的基質反應較強而黑殭菌195則只有中度的反應。就繼代培養而言，白殭菌第九代之培養對phosphatase alkaline, leucine aminopeptidase, α-galatosidase, β-galactosidase的基質反應活性會下降。蠟蚧幹枝孢菌第六代之培養對leucine aminopeptidase, valine aminopeptidase, phosphoamidase, β-glucosidase，及β-glucosaminidase反應活性會下降。相反的對phosphatase alkaline 反應有些微上升。以上的結果顯示API ZYM測試方法可提供快速的半定量的方法來辨識不同分離株及繼代培養的酵素活性。

Key words: Beauberia bassiana, Metarhizium anisopliae, Verticillium lecanii, API ZYM.

關鍵詞: 白殭菌、黑殭菌、蠟蚧幹枝孢菌、API ZYM

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Enzymatic Characterization of Three Entomopathogenic Fungi with the API ZYM System

Wen-Feng Hsiao Department of Plant Protection, National Chiayi Institute of Technology, Chiayi, Taiwan, R.O.C.

ABSTRACT

The enzymatic profiles of *Beauveria bassiana* (Bb), *Metarihizium anisopliae* (Ma), *Verticillium lecanii* (Vl), and their subcultures were studied by using the API ZYM system. Isolates all showed no reaction to substrates with β-glucuronidase, or cystein aminopeptidase except for Ma (str. 195). All isolates showed little activity or were completely without activity to substrates with lipase, trysin, chymotrysin, α-glucosidase, or α-fucosidase. However, Bb Ho 42 reacted strongly to substrate with α-mannosidase.

Both isolates of *M. anisopliae* (strs. 194 and 195) reacted strongly to substrates with leucine aminopeptidase, acid phosphatase, phosphoamidase, or β-glucosaminidase. Additionally, *M. anisopliae* str. 194 reacted strongly to the substrates with esterase (C_4), or esterase lipase, while *M. anisoplaie* str. 195 moderately reacted to the substrates with β-glucosidase.

Results showed that the enzymatic activity of *B. bassiana* against substrates with phosphatase alkaline, leucine aminopeptidase, α-galactodase, or α-galactodase declined after serial passages from subcultures of Ho. 212 F_3 to F_9. *V. lecanii* exhibited the tendency of declining activity against substrates with leucine aminopeptidase, valine aminopeptidase, phosphosamidase, β-glucosidase, or β-glucosaminidase from subcultures of F_4 to F_6. On the other hand, the reaction to the substrate with phosphatase alkline increased. The results of this study indicate that API ZYM techniques provide a rapid and reproducible semi-quantitative method for revealing enzymatic activity.

Key words: *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii,* API ZYM.

Introduction

Fungal diseases in insects are common and widespread, and may cause the collapse of insect populations under proper weather conditions. Among the entomopathogenic fungi, *Beauveria bassiana, Metarhizium anisopliae*, and *Verticillium lecanii* are presently being studied extensively for their developement in insect pest control. Previous studies have demonstrated that extracellular enzymes or secondary metabolites secreted by entomopathogens play an important role

in the pathogenicity toward insects (Hajek and St. Leger, 1994). Hsiao and Khachatourians (1997) and St. Leger *et al.* (1986) have used API ZYM to determine enzymatic profiles and found it to be a rapid and reproducible method for revealing enzymatic activity differences of fungal isolates and UV-induced mutants. Therefore, in this study, the API ZYM system was employed to determine enzymatic activities of *B. bassiana, M. anisopliae*, and *V. lecanii* either in stock culture or after a series of subcultures.

The fungal isolates, *Beauveria bassiana* (Cols. 41, 42 and Ho. 212), *Metarhizium anisopliae* (strs. 194, 195), and *Verticilium lecanii* (Ho. 159), were obtained from Professor S. S. Tzeng (National Taiwan Unversity, Taiwan). These 3 isolates were cultured on YPDA medium and incubated at 25°C (12L: 12D) for 7d; the harvested conidia were suspended in 0.05% Tween 80 at a concentration of 10^7 conidia / ml, and stored in a refrigerator at 4°C before use. A series of subcultures of *B. bassiana* (Ho. 212) and *V. lecanii* (Ho. 159) were also tested for their enzymatic activity changes.

To determine the enzymatic activity, the API ZYM gallery was placed in a tray, supplemented with ca. 5 ml distilled water to provide a humid atmosphere, and 100 ul of spore suspension was pipetted in each cupule of the gallery, respectively. After inoculation, the tray was covered with a plastic lid, and incubated at 25°C for 3d. After incubation, 1 drop of each of reagent ZYM A and reagent ZYM B were individually added to each cupules respectively. After 5 min, the color developed and then was compared with the colorchart. A value reading ranging from 0 to 5 was assigned corresponding to the color developed according to the color chart. Zero corresponds to a negative reaction, and 5 to a maximum reaction, and values 1-4 represent intermediate reactions.

Tabel 1 shows the enzymatic profiles of the tested entomopathogens. All isolates showed no reaction to substrates with β-glucuronidase, or cystein aminopeptidase except Ma (str. 195). Isolates were only a little active or completely inactive to substrates with lipase, cysteine aminopeptidase, trysin, chymotrysin, α-glucosidase, or α-fucosidase. Bb Col. 42 is the only isolate which reacted strongly to substrate with α-mannosidase.

Both isolates of *M. anisopliae* (strs. 194 and 195) showed strong reactions to substrates with leucine aminopeptidase, phosphatase acid, phosphoamidase, or β-gl ucosaminidase. Besides, *M. anisopliae* str. 194 reacted strongly to substrates with esterase (C_4), esterase lipase, or β-glucos idase, while *M. anisopliae* str. 195 reacted moderately to the above substrates.

The activity of subculture F_9 of *B. bassiana* started to decline to substrates with phosphatase alkaline, leucine aminopeptidase, α-galactosidase, or β-galactosid ase. Likewise, subculture F_6 of *Verticillium lecanii* started to decline for substrates with leucine aminopeptidase, valine aminopeptidase, phosphoamidase, β-glucosidase, or β-glucosaminidase. On the contrary, the activity of *V. lecanii* to the substrate of phosphatase alkaline has increased.

The API ZYM pattern of *M. anisopliae* differed from that of the other entomopathogenic species with respect to higher activity against both caprylate and 6-Br-2-naphtyl-D-glucopyranoside. The result differed from that of St. Leger *et al.* (1986), and needs futher study.

The results of this study indicate that the API ZYM techniques provide a rapid and reproducible semi-quantitative method for revealing enzymatic activity of different isolates of entomopathogenic fungi. St. Leger *et al.* (1986) indicated that API ZYM might potentially be used to identify and characterize isolates patented for commercial products. In this study, API ZYM also documented fluctuations of enzymatic activities between

Table 1. Enzymatic activities of isolates of 3 entomopathogenic fungi revealed by the API ZYM system[b].

Isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
B. bassiana																				
Ho 41	0	3	2	1	0	3	1	0	0	0	2	3	0	4	0	1	3	4	0	<1
Ho 42	0	3	3	1	0	3	1	0	0	0	3	3	<1	4	0	1	3	4	5	<1
Ho 212 F_3[a]	0	3	2	3	<1	1	<1	0	0	0	3	3	<1	2	0	0	3	3	<1	0
Ho 212 F_6[a]	0	1	2	3	<1	<1	<1	0	0	0	3	3	<1	2	0	0	3	3	<1	0
Ho 212 F_9[a]	0	1	3	3	<1	0	<1	0	0	<1	3	3	0	<1	0	0	3	3	<1	0
M. anisopliae																				
str. 194	0	2	4	5	0	5	<1	0	<1	<1	5	4	<1	<1	0	0	5	4	<1	0
str. 195	0	1	3	3	1	5	1	1	<1	0	5	5	<1	<1	0	1	3	4	<1	<1
V. lecanii Ho 159																				
F_4[a]	0	2	3	3	1	5	2	0	1	<1	5	3	0	<1	0	0	3	3	1	<1
F_5[a]	0	3	3	3	0	1	0	0	0	0	5	3	1	<1	0	0	3	3	0	0
F_6[a]	0	4	3	3	0	<1	0	0	0	0	4	<1	0	<1	0	0	1	2	0	0

[a] A series of cultures from the stock culture; F_3 is subcultured 3 times.

[b] Tests: (1)control, (2) phosphatase alkaline, (3) esterase (C_4), (4) esterase lipase (C_8), (5) lipase (C_{14}), (6) leucine aminopeptidase, (7) valineamino-peptidase, (8) cysteineaminopeptidase,(9) trypsin, (10) chymotrypsin, (11) phosphatase acid, (12) phosphoamidase, (13) α-galactosidase, (14) β-galactosidase, (15) β-glucuronidase, (16) α-glucosidase, (17) β-glucosidase, (18) β-glucosaminidase, (19) α-mannosidase, (20) α-fucosidase. Number value from 0 to 5 indicates increasing color intensity.

parent strains and serial subcultures offspring of entomopathogens.

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以 API ZYM 系統測定蟲生真菌之酵素特性

蕭文鳳　國立嘉義技術學院　植物保護科　嘉義市學府路300號

摘　要

本實驗利用半定量之API ZYM系統來研究三種蟲生真菌，白殭菌*Beauveria bassiana*，黑殭菌*Metarhizium anisopliae*，蠟蚧幹枝孢菌*Verticillium lecanii*的酵素剖圖。所有的分離株對β-glucuronidase及cysteine aminopeptidase的基質無反應，但黑殭菌195菌株對後者有反應。所有的分離株對Lipase, trypsin, chymotrypsin, α-glucosidase及α-fucosidase的基質無反應或只有輕微的反應。而白殭菌Col 42品系對α-mannosidase基質有很強的反應。

黑殭菌的二個分離株(194及195) 對leucine aminopeptidase, phosphatase acid, phosphoamidase, β-glucosaminidase具有強烈之反應。除此外，黑殭菌194對esterase, esterase lipase和β-glucosidase的基質反應較強而黑殭菌195則只有中度的反應。就繼代培養而言，白殭菌第九代之培養對phosphatase alkaline, leucine aminopeptidase, α-galatosidase, β-galactosidase的基質反應活性會下降。蠟蚧幹枝孢菌第六代之培養對leucine aminopeptidase, valine aminopeptidase, phosphoamidase, β-glucosidase，及β-glucosaminidase反應活性會下降。相反的對phosphatase alkaline反應有些微上升。以上的結果顯示API ZYM測試方法可提供快速的半定量的方法來辨識不同分離株及繼代培養的酵素活性。

關鍵字：白殭菌，黑殭菌，蠟蚧幹枝孢菌，API ZYM。