Carbon Metabolism of *Beauveria bassiana* May be Related to Virulence Against *Plutella xylostella* (Lep.: Plutellidae) and *Leptinotarsa decemlineata* (Col.: Chrysomelidae)

R. Talaei-Hassanloui[1] , A. Kharazi-Pakdel [1] , M. S. Goettel [2] , J. Mozaffari [3] and J. Bissett[4]

Abstract

Fermentation of 49 carbohydrates and oxidation and apssimilation of 96 substrates were examined for ten *Beauveria bassiana* isolates. Principal component analysis indicated that there are significant differences among isolates for utilization of some substrates. Esculine and D-glucose were positively fermented by all isolates. Isolates were grouped into four clusters on the basis of hierarchial analysis of 96-hr reaction data. Using canonical variate analysis, it was determined that N-acetyl-D-glucosamine fermentation and β-cyclodextrin oxidation were strongly and positively correlated with pathogenicity of isolates against diamondback moth, *Plutella xylostella* and Colorado potato beetle, *Leptinotarsa decemlineata*. In contrast, Sebacic acid assimilation was negatively correlated to virulence against these insects.

Key words: *Beauveria bassiana*, carbohydrate utilization, virulence, sebacic acid, β-cyclodextrin, N-acetyl D-glucosamine

Introduction

Despite many advances in pest control, including the transgenic expression of insect toxic proteins in crop plants, insects continue to represent serious competition for food globally (1). Furthermore, insect resistance to chemical insecticides (2), to *Bacillus thuringiensis* and to Bt-transgenic crops (13) suggests that crop protection alternatives should be exploited. One of these alternatives is the utilization of entomopathogenic fungi, as one of the most important factors regulating insect populations in nature (1). *Beauveria bassiana* , the most common and ubiquitous fungal entomopathogen (7), has a wide host range but differences in both virulence and host specificity among isolates have been reported (4, 8).

1- Department of Plant Protection , College of Agriculture, University of Tehran, Karaj, 31587-11167, Iran
2- Lethbridge Research Center, 5403-1 Avenue South, P.O. Box 3000, Lethbridge, Alberta T1J 4B1, Canada
3- Department of Genetics, Seed and Plant Improvement Institute, Karaj, Iran
4- Easter Cereal and Oilseed Research Centre, Ottawa ,Ontario, K1A 0C6, Canada

Several strains of *B. bassiana* have been classified by agglutination and immunochemical methods (12, 14), estrase tests and ability to utilize hydrocarbons (6, 9, 10). However, these studies did not attempt to relate these traits to virulence against insect hosts. .

The outermost insect cuticular layer is the epicuticle, covered by a thin layer of lipids, among which usually prevail long-chain hydrocarbons conforming a highly inert coating. This provides a barrier against water loss and chemical or microbial invasion. The biochemical mechanisms involved in the utilization of very-long-chain hydrocarbons, of chain lengths similar to those present in the insect epicuticle, and the uptake and transport of the hydrocarbons and their metabolites into the microorganisms are still poorly understood.

The purpose of this study was to determine if we could find a relationship between virulence and ability to metabolize specific substrates. We analyzed variation in carbon metabolism of local fungal isolates of *B. bassiana* using API 50CH strips and Biolog FF MicroPlates™, looking for any possible correlation between these variables and virulence against diamond back moth (DBM), *Plutella xylostella* L. and Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say.

Materials and Methods

Isolates

Ten *B. bassiana* isolates, which had been obtained from different hosts and geographical locations (table 1), were plated onto SDAY (Sabouraud Dextrose Agar +1% yeast extract) and incubated at 25° C and 16:8 h (light: dark) until sufficient conidiation developed for inoculation of the API 50CH strips and Biolog microplates (approximately 10 days).

Fermentation of substrates

The API 50CH strip (Biomerieux SA, Durham, NC) comprises 50 microtubes each containing an anaerobic zone and an aerobic zone. The first tube contains no substrate and is used as a negative control. The remaining tubes contain different compounds as carbon sources. Tubes containing API-CHB mediump (Biomerieux Inc.) were inoculated with conidia and vortexed for 5 seconds. Each microtube in the API 50CH strip was inoculated with 200µl of the conidial suspension. The strips were incubated at the same condition as fungal cultures, and fermentation of substrates recorded after 48, 72 and 96 h incubation according to the manufacturer's instructions.

Oxidation and assimilation of substrates

The biolog system is a useful method of sub-typing species of microorganisms by measuring cellular metabolism using carbon source tracking. The Biolog FF MicroPlate™ (Biolog Inc., Hayward, CA) contains 96 wells, of which 95 containing pre-selected carbon sources, necessary nutrients, and tetrazolium violet. Iodo-nitrotetrazolium (INT) is reduced by the activity of succinate dehydrogenase in the citric acid cycle to an insoluble red-colored formazan salt with peak absorbance near 490 nm. Absorbance readings at 490nm reflect respiratory activity, and readings at 750 nm measure mycelial production and substrate assimilation.

Table 1. The accession numbers, original host, geographical origin and virulence of *B.bassiana* isolates against DBM and CPB

Accession Number	Host or Source	Country	%Mortality ±SE[d]	
			DBM	CPB
DEBI007	Soil[a]	Iran- Tehran	25.7±1.6	26.1±1.9
DEBI008	*Chorthippus brunneus* (Orth.: Acrididae)	Iran- Tehran	31.3±2.4	36.6±3.1
DEBI003	*Rhynchophorus ferrugineus* (Col.: Curculionidae)	Iran- Saravan	28.2±1.2	33.7±1.8
DEBI002	Soil[a]	Iran- Atashgah	17.9±1.9	25.8±1.3
DEBI001	Soil[a]	Iran- Fashand	32.6±1.1	42.6±1.8
KCF106	*Chilo suppressalis* (Lep.: Pyralidae)	Iran- Rasht	41.6±3.0	74.1±2.7
KCF107	Soil[b]	Iran- Karaj	53.8±1.0	58.5±2.9
GHA	Mycotrol WP[c]	USA	40.2±2.0	50.1±1.6
LRC107	*Leptinotarsa decemlineata* (Col.: Chrysomelidae)	Portugal	22.6±1.7	36.7±2.2
LRC137	*L. decemlineata*	Canada	15.3±1.0	26.6±1.1

DEBI isolates were kindly supplied by Mehran Ghazavi, Plant Pests and Diseases Research Institute, Tehran, Iran. KCF isolates from Dept. of Plant Protection, University of Tehran. LRC isolates held at the Lethbridge Research Centre. Single spore of these isolates was developed and used in assays.

[a] Isolated from soil using *Galleria mellonella* (Lepidoptera: Pyralidae) larvae as insect bait.

[b] Isolated from soil using selective medium DOC2 (11).

[c] Mycotech GHA was isolated from Mycotrol® WP. The GHA strain was developed from a reisolation of strain ARSEF 201, originally from *Diabrotica undecimpunctata* (Coleoptera: Chrysomelidae) from Corvallis, OR.

[d] Means of three and four bioassays on DBM and CPB, respectively . Mortality data have been achieved from assays with spraying 2200 co./ cm^2 concentration of each isolate against the second instar larvae of DBM and CPB.

Conidia were collected by rolling a sterile cotton swab over areas of conidiation and dispersing them in a sterile inoculating fluid composed of a gelling agent (0.25% phytagel) and surfactant (0.03% Tween 40) in distilled water. A uniform spore concentration was

achieved by adjusting the absorbance of the suspension to 75±2% at 490 nm using a turbidimeter. One hundred microlitres of suspension was inoculated into each of the 96 wells of the microplate. Microplates were incubated at 26˚C in the dark, and absorbance readings at 490 nm (respiration) and 750 nm (assimilation) recorded using a microplate reader after 24, 48, 72 and 96 hrs.

Statistical analysis

Substrates with differing fermentations, oxidations and assimilations among the 10 isolates (P<0.05) were selected for multivariate analyses on the basis of 96 hr data (SPSS 10). A dendrogram of biochemical relatedness was constructed using the UPGMA (unweighted pair-group method using arithmetic averages) clustering method (SYSTAT 10). Stepwise regression (Minitab 13.1) was used to determine which substrates could be related to virulence (Talaei-Hassanloui *et al*., unpublished). Associations between virulence of isolates and their differing abilities to utilize C-substrates were determined through canonical variate analysis (CVA), and corresponding correlations calculated using the Pearson correlation coefficient (r) and Bonferroni probability (P) using SYSTAT 10.

Results

Fermentation of substrates

The isolates differed in their utilization of the carbohydrate substrates. Esculine and D-glucose were metabolized by all isolates, whereas none of the isolates was able to utilize the carbohydrates α-methyl-D-mannoside, α-methyl-D-glucoside, D-tagatose, L-fucose, D-fucose, 5-ceto-gluconate and 2-cetogluconate.

Principal component analysis of API 50CH data indicated that ability to utilize substrates salicine, D-mannose, D-raffinose, ribose, sorbitol, amygdaline, lactose, D-xylose, dulcitol, inositol, N-acetyl-D-glucosamine and arbutine was highly variable among the isolates. A dendrogram based on the metabolic data grouped the isolates into four metabolic strains (figure 1).

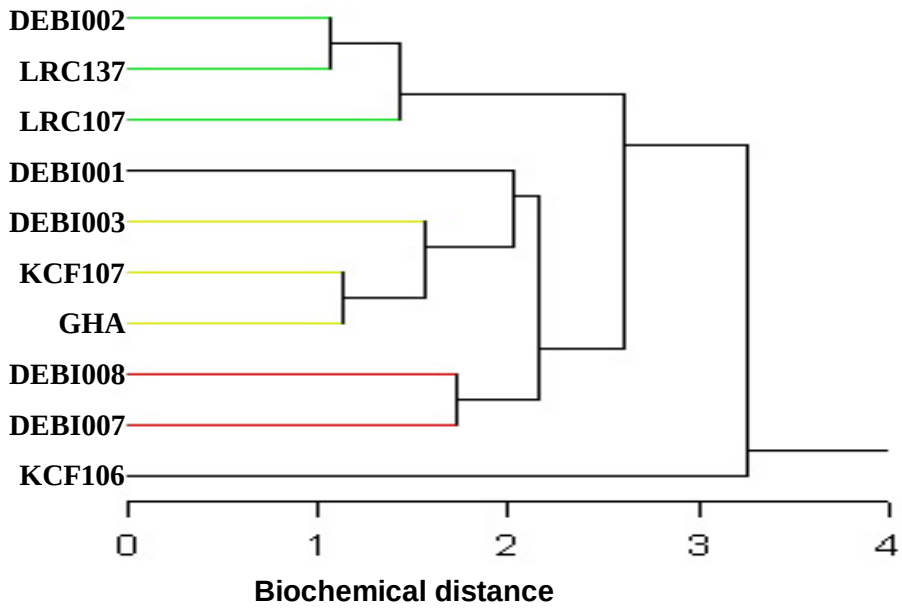

Figure 1. Dendrogram representing the relationship of 10 isolates of *B. bassiana* on the basis of rate of fermentation of carbohydrates.

The first cluster consisted of three isolates DEBI002, LRC 137 and LRC107. Isolates DEBI001, DEBI003, KCF107 and GHA were placed into the second cluster. The third cluster included isolates DEBI007 and DEBI008. Isolate KCF106 was the only member of a fourth cluster. Stepwise regression revealed that fermentation of N-acetyl glucosmine, maltose, xylitol, lactose and D-arabitol were related to virulence of isolates against *P. xylostella* and *L. decemlineata* but only N-acetyl-D-glucosamine ($F=38.12$, $P<0.001$) fermentation was significantly correlated to virulence. This correlation was strong and positive (figure 2)

Respiratory activity on carbohydrates

There was limited variability among isolates in their ability to oxidize C-substrates (490 nm readings in Biolog microplates). The 10 isolates were clustered into two groups in UPGMA cluster analysis (figure 3). The first group consisted of DEBI001, GHA, KCF107, DEBI008, DEBI007, LRC107, DEBI003 and DEBI002 and the second included isolates KCF106 and

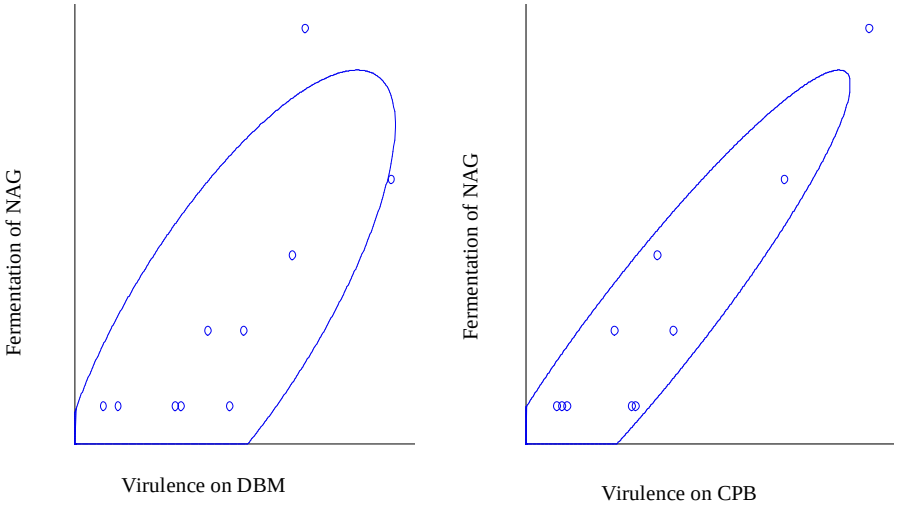

Figure 2. Correlation between fermentation ability of isolates on N-acetyl-D-glucosamine (NAG) and their pathogenicity against DBM (r = 0.78; Bonf., *P*= 0.008) and CPB (r = 0.95; Bonf., *P*=0.00)

LRC137. Oxidation of β-cyclodextrin, L-phenylalanine and L-alanylglycine were found to be associated with the isolates that exhibited virulence against DBM and CPB. Canonical variate analysis indicated that only oxidation of β-cyclodextrin (F=9.83, *P*<0.02) was significantly and positively correlated to virulence of isolates against these two insect species (figure 4).

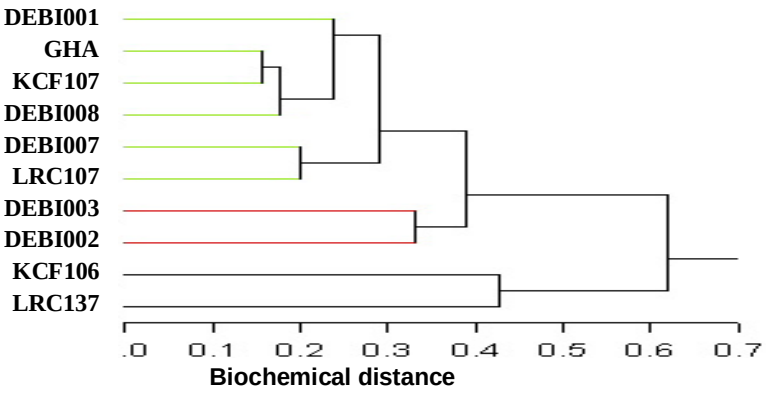

Figure 3. Dendrogram representing grouping of 10 isolates of *B. bassiana* in cluster analysis based on respiratory activity on carbohydrates

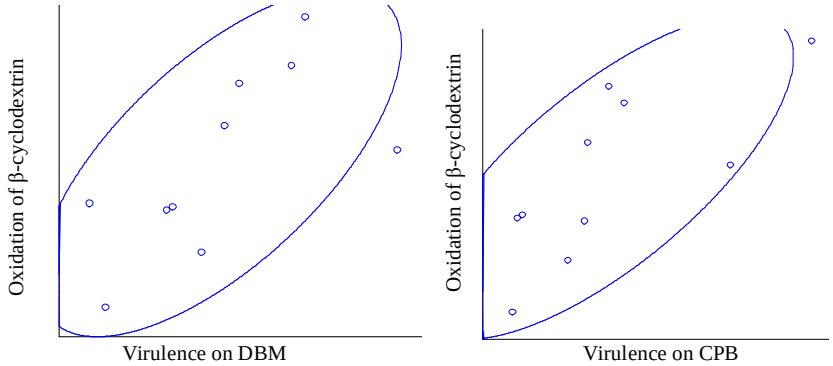

Figure 4. Correlation between respiratory activity of isolates on β-cyclodextrin and their virulence against DBM (r = 0.67; Bonf., *P*= 0.03) and CPB (r = 0.8; Bonf., *P*=0.00).

Assimilation of substrates (750 nm data).

Although 11 metabolic variables had high variability among the 10 isolates, UPGMA could not cluster them as different strains. Canonical variate analysis revealed that the assimilation of Sebacic acid (750 nm readings) was significantly (F=11.36, *P*<0.01) and negatively correlated to virulence of isolates against DBM and CPB (figure 5).

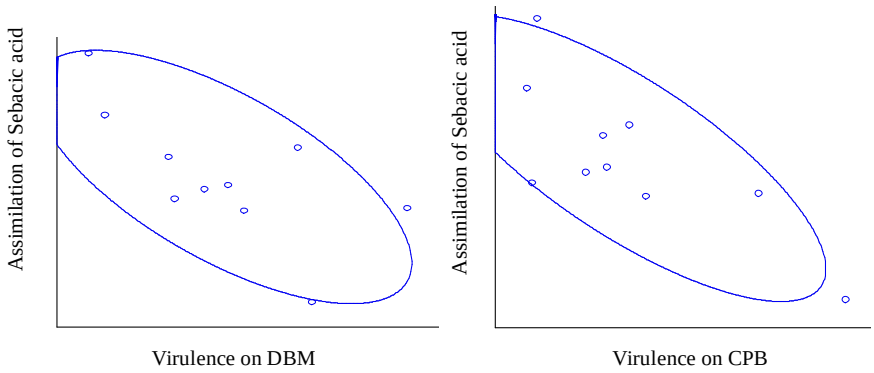

Figure 5- Correlation between assimilation of Sebacic acid and virulence of isolates against DBM (r= -0.68; Bonf., *P*= 0.031) and CPB (r = -0.73; Bonf., *P*=0.015)

Discussion

Of approximately ten fungal species currently being developed or utilized for insect control, *Metarhizium anisopliae* and *B. bassiana* have received most attention as far as molecular and biochemical approachs toward understanding mechanisms involved in infection processes are concerned. Insect cuticular hydrocarbon degradation has been shown to be a major metabolic pathway in entomopathogenic fungi, related to peroxisomal proliferation (3). Evidence for a metabolic shift in carbon assimilation to fungal lipids after hydrocarbon growth induction was shown by Juarez *et al*. (6). API 50CH strips and Biolog microplates were selected for the evaluation of variation in carbon metabolism among the isolates, because of their availability and versatility. Among the 10 isolates of *B .bassiana* tested, a variation in carbon metabolism was found. Ninety-six hour treatment of carbohydrate fermentation clustered the isolates into four strains. The first strain contained isolates DEBI002 (soil origin, Iran- Atashgah), LRC137 (CPB origin, Canada) and LRC 107 (CPB origin, Portugal), of which 2 were isolates with low virulence to DBM and CPB. Half of the isolates in the second strain cluster and all of the isolates in the third cluster were highly and moderately virulent respectively. We also found a positive and strong correlation between fermentation of N-acetyl-D-glucosamine and pathogenicity of isolates against DBM and CPB. In other words, the more fermentation ability of a specific isolate on N-acetyl-D-glucosamine, the higher the virulence against these two insect species. Since N-acetyl glucosamine is a chitin monomere, and chitin is a polysaccharide constituent of insect cuticle, this correlation could be expected. To our knowledge, this is the first report on the detailed correlation between virulence of *B. bassiana* and its ability in metabolizing defined substrates. Fermentation of N-acetyl-D-glucosamine, oxidation of β-cyclodextrin and assimilation of Sebacic acid were found as possible indices in evaluating potential virulence of *B.bassiana* isolates against DBM and CPB. There was no details regarding possible role of β-cyclodextrin in insect physiology in references except that it has been reported that β-cyclodextrin facilitates cholesterol efflux from fat body and midgut *Manduca sexta* (L.) (Lep.: Sphingidae) larvae *in vitro* (5), which it doesn't have any comparable point with our mentioned result.

Variation in biochemical profile among *M. anisopliae* isolates was reported by Rath *et al*. (10), but they just used this characteristic as a chemotaxonomic trait to cluster *M. anisopliae* isolates and discriminate them from *B. bassiana* and *Microhilum oncoperae* . In our study, clustering of *B. bassiana* isolates based on metabolic characters did not reflect the host or

geographic origin for the strains studied. Research on a larger set of strains representing more diverse hosts is required to test the hypothesis generated in the current study regarding correlations between carbohydrate metabolism and virulence in *B. bassiana*.

Acknowledgements

This publication is a part of the first author's Ph.D. thesis funded by the University of Tehran; which we thank for the financial support. We are also grateful to Grant Duke for his technical assistance.

References

1- Bidochka, M., A. Kamp & J. DeCroos, 2000. Insect pathogenic fungi: from genes to populations.In: Fungal pathology, J.W. Kronstad (ed.) pp. 171-193. Kluwer Academic Publishers.

2- Charlesworth, B. ,1998. Adaptive evolution: the struggle for dominance. Current Biology, 8: 502-504.

3- Crespo, R., M. P. Juarez & L. Cafferatta, 2000. Biochemistry of the interaction between entomopathogenous fungi and their insect host-like hydrocarbons. Mycologia, 92(3): 528- 536.

4- Ferron, P., J. Farques & G. Riba, 1991. Fungi as microbial insecticides against pests. In *Hand book of Applied Mycology: Humans, Animals and insects.* Arora, D.K., Mukherji, K. G. and Drouhet, E. (eds.) Vol. 2, Marcel Dekker. New York, pp. 665-705.

5- Jouni, Z., B. McGill & M. Wells, 2002. Beta-cyclodextrin facilitates cholesterol efflux from larval *Manduca sexta* fat body and midgut in vitro. Biochemical and Molecular Biology, 132(4): 699-709

6- Juarez, M. P., R. Crespo, G. C. Fernandez, R. Leucona & F. R. Cafferata, 2000. Characterization and carbon metabolism in fungi pathogenic to Triatoma infestans, a chagas disease vector. Journal of Invertebrate Pathology, 76: 198-207.

7- Leathers, T.D., S. C. Gupta & N. J. Alexander, 1993. Mycopesticides: status, challenges and potential. Journal of Industrial Microbiology, 12: 69-75.

8- McCoy, C. W., R. A. Samson & D. G. Boucias, 1988. Entomogenous fungi: In *CRC Handbook of Natural Pesticides*. Volume V: Microbial Insecticides. Part A..

Entomogenaus protozoa and fungi, eds. Lgnoffo, C. M. & N. B. Mandava, CRC Press, Boca Raton, pp. 151-236..

9- Mugnai, L., P. Bridge & H. Evans, 1989. A chemotaxonomic evaluation of the genus *Beauveria.* Mycological Research, 92(2): 199-209.

10- Rath, A.C., C. J. CARR & B. R. Graham, 1995. Characterization of *Metarhizium anisopliae* strains by carbohydrate utilization (API50CH). Journal of Invertebrate pathology 65, 152-161.

11- Shimazu, M. & H. Sato, 1996. Media for selective isolation of an entomogenous fungus, Beauveria bassiana (Deuteromycotina: Hyphomycetes). Applied Entomology and Zoology, 31(2): 291-298.

12- Shimizu, S. & K. Aizawa, 1988. Serological classification of Beauveria bassiana. Journal of Invertebrate Pathology, 52: 348-353.

13- Tabashnik, B. E., Y. Liu, T. Malvar, D. G. Heckel, L. Masson, V. Ballester, F. Graner, J. L. Mensua & J. Ferre, 1997. Global variation in the genetic and biochemical basis of diamondback moth resistance to *Bacillus thuringiensis* , Proceeding of National Academic Science, USA, 94: 12780-12785.

14- Tan, Y. & A. Ekramoddoullah, 1991. Immunochemical characterization of the entomopathogenic fungus *Beauveria bassiana.* Journal of Invertebrate Pathology, 57: 269-276.

نامه‌ی انجمن حشره‌شناسی ایران، ۲۵: (۱)، ۱۳۸۴

متابولیسم کربن در *Beauveria bassiana* و ارتباط احتمالی آن با زهرآگینی جدایه‌ها روی بید کلم *Plutella xylostella* (Lep.: Plutellidae) و سوسک کلرادوی سیب زمینی *Leptinotarsa decemlineata* (Col.: Chrysomelidae)

رضا طلایی حسنلویی[۱]، عزیز خرازی پاکدل[۱]، مارک اس گوتل[۲]، جواد مظفری[۳] و جان بیست[۴]

چکیده

ده جدایه از قارچ بیمارگر حشرات *Beauveria bassiana* از نظر توانایی تخمیر ٤٩ منبع کربوهیدراتی در نوارهای API 50CH و اکسیداسیون و جذب و ساخت ٩٦ سوبسترا در میکروپلت‌های Biolog، مطالعه و مقایسه شد. تجزیه‌ی داده‌ها نشان داد که جدایه‌ها در مصرف بعضی سوبستراها، اختلاف معنی‌داری دارند. پاسخ تخمیری تمام جدایه‌ها برای اسکولین و D– گلوکز مثبت بود. تجزیه‌ی مراتبی داده‌های ٩٦ ساعته‌ی تخمیر، جدایه‌ها را در چهار کلاستر قرار داد که جدایه‌ی KCF106 دارای بیشترین فاصله بیوشیمیایی با سایر جدایه‌ها بود. بر اساس تجزیه‌ی همبستگی متعارف، مشخص گردید که تخمیر N– استیلD– گلوکز آمین و اکسیداسیون β – سیکلودکسترین با بیماری‌زایی جدایه‌ها روی بید کلم، *Plutella xylostella* و سوسک کلرادوی سیب زمینی، *Leptinotarsa decemlineata* دارای همبستگی مثبت و قوی است. در مقابل جذب و ساخت سوبسترای سباسیک اسید با بیماری‌زایی جدایه‌ها روی این دو حشره، همبستگی منفی نشان داد.

واژه‌های کلیدی: *Beauveria bassiana*، مصرف کربوهیدرات، زهراگینی، سباسیک اسید ،β – سیکلودکسترین، N – استیل D– گلوکوزامین

۱– گروه گیاه‌پزشکی دانشکده‌ی کشاورزی دانشگاه تهران، کرج
۲– مرکز تحقیقات لتبریج، صندوق پستی ۳۰۰۰، آلبرتا، کانادا
۳– گروه ژنتیک، موسسه‌ی اصلاح و تهیه بذر و نهال، کرج
۴– مرکز تحقیقات غلات و دانه‌های روغنی، آتاوا، آنتاریو، کانادا
این مقاله در تاریخ ۱۳۸۴/۱/۳۰ دریافت و چاپ آن در تاریخ ۱۳۸۴/۴/۲۵به تصویب نهایی رسید.