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UTILIZATION OF CARBOHYDRATES BY *BEAUVERIA BASSIANA* ISOLATES OBTAINED FROM FOREST PESTS

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Abstract: Carbohydrate utilization profiles of ten isolates of the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuillemin were studied and compared to assist in determining their phenotypic characterization. Isolates were obtained in pure cultures from dead individuals of several forest pests collected from different regions in Bulgaria. Studies on utilization profiles were based on the acidification of twenty carbohydrates.

The results indicate that sucrose, maltose and trehalose were assimilated at a high degree compared to esculin, arabinose and dulcitol. According to the results of a cluster analysis of the carbohydrate utilization profiles, the *B. bassiana* isolates were divided into two larger groupings. All isolates in the first larger cluster were obtained from the coleopteran insects – *Stenomax aeneus*, *Ips typographus*, *I. sexdentatus* and *Dryocoetes autographus*. Isolates from the other cluster were obtained from the lepidopteran larvae of *Thaumetopoea pityocampa* and *Lymantria dispar,* and from an adult of the coleopteran species *Hylurgops palliatus*. We determined that each *B. bassiana* isolate exhibited a different and specific carbohydrate utilization profile but differences at the p-level < 0.05 were significant among some of them. The most distinguishable was the isolate 560Bb obtained from *T. pityocampa*. Differences between the isolate 560Bb and the other nine *B. bassiana* isolates were highly significant at the p-level < 0.005. Isolate 433Bb obtained from a dead adult of *I. typographus* was significantly different from five of the studied isolates at the p-level < 0.05.

Key words: entomopathogenic fungi, *Beauveria bassiana*, carbohydrate utilization, forest pests

INTRODUCTION

The entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuillemin (anamorph Ascomycota, Hypocreales, Cordycipitaceae) is a cosmopolitan species frequently isolated from dead individuals of different arthropod species in agricultural and forest biocoenoses. Many of these isolates have been maintained in microbial collections. Their phenotypic characteristics have been studied for species identification purposes. They have also been studied to assist in investigations to determine the proper cultural media for cultivating these organisms for use as biological control agents.

Variability in carbon requirements among different *Beauveria* species as well as among different *B. bassiana* isolates was reported by Campbell *et al*. (1983), Draganova (1997), Draganova and Lecheva (2002), Sun and Liu (2006). It was established that in general, monosaccharides and disaccharids dextrose, mannose, sucrose, maltose and trehalose were suitable for *B. bassiana* growth and sporulation. Rhamnose, D-sorbose, lactose, inulin and D-arabinose were carbohydrates on which the fungus grew and sporulated least.

Biochemical methods for identification purpose are well-known in bacteriology (Lanyi 1987). Release of organic acids by bacterial strains which is a result of carbohydrate catabolism, has been demonstrated with pH indicators. Substantial amount of acid produced by rapidly fermenting bacteria can be detected with colour changing indicators at pH 5.0–5.5 (Andrade's indicator, bromcresol purple). To show acid production by weak fermenters and oxidizers, more sensitive indicators with pH range of 6.0–8.0 (bromthymol blue, phenol red) were used.

Biochemical properties and in particular their utilization of carbohydrates can be used to support the morphology and are useful for identifying species of *Beauveria*.

Mugnai *et al*. (1989) studied the intra- and interspecific variation of 32 isolates assigned to the genus *Beauveria*. They concluded that cultural characters were highly variable and could not be used reliably to separate species. However, spore form was the most useful criterion for distinguishing between species. The use of biochemical data generally supports species concepts based purely on morphology, with the exception of *B. bassiana* which comprises a heterogenerous assemblage of strains.

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The API ZYM[tm] system, which utilizes colour reactions to determine enzyme activity, was used to characterize entomopathogenic fungi by St. Leger *et al*. (1986) and El-Sayed *et al*. (1992). This test was used by Bridge *et al*. (1993) to identify and distinguish among *Metarhizium* spp., and by Rath *et al*. (1995) to distinguish between two *Metarhizium* species, *B. bassiana* and *Microhilum oncoperae* Yip & Rath. The carbohydrate utilization profiles of the two *B. bassiana* isolates also differed in their morphology, protein profiles and pathogenicity against *Leptinotarsa decemlineata* Say and *Coleomegilla maculata lengi* Timb. (Todorova *et al*. 1994). Based on data obtained on the reactions of strains of *Beauveria* and *Tolypocladium* spp. to carbohydrates, Todorova *et al*. (1998) proposed the use of an identification key to complement the morphological description of the species.

Pernfuss *et al*. (2003) used the BIOLOG™ microtiter plate procedure to test strains of *Beauveria* spp. for their metabolization of 130 carbon sources and were able to distiguish between *B. brongniartii* (Sacc.) Petch and *B. bassiana*.

The objective of our research was to study and compare carbohydrate utilization profiles of *B. bassiana* isolates obtained from several forest insect pests in Bulgaria in order to complement their phenotypic characterization.

MATERIALS AND METHODS

Ten *B. bassiana* isolates used in the study were recovered in pure cultures from dead insects collected between 2006–2009. The insects were collected from natural populations of forest pests from different regions in Bulgaria. The identity and origin (initial hosts) of the isolates are shown in table 1. Isolates are maintained in the collection of entomopathogenic fungi at the Department of Biological and Integrated Pest Control (Plant Protection Institute, Bulgaria) as stock cultures on slopes of SDAY (Sabouraud dextrose agar with yeast extract) in tubes at 4±1°C subcultured on the same media ones per year.

Isolates 433 and 434 had been subcultured from the stock cultures two times before this study, the other isolates – once.

Isolates were cultured on SDAY in tubes for 15 days at 25±1°C. Conidia that were produced were suspended in water and suspensions were diluted to $1x10^8$ conidia/ml after determination of the concentrations by haemocytometer.

Liquid cultural media (produced by BulBio, Infectious Diseases Institute, Bulgaria) containing 1% solution of one of twenty carbohydrates (monosaccharides, disaccharides, trisaccharides, polyhydric alcohols, polysaccharides, glycosides) and Andrade's indicator were used to examine the biochemical profiles of the fungal isolates. The experiments were carried out following the manufacturer's recommendations. Conidial suspensions (0.1 ml) were dropped into the tubes with carbohydrate solutions. Cultures were incubated for five days at 25±1°C in darkness, to avoid the influence of light on the indicator. Results of the acidification caused by released organic acids after the catabolism of the respective carbohydrate, were checked visually, for the as degree of colour changes in the medium. Colours ranged from light yellow (negative reaction – 0 degrees acidification) to deep purple (maximum acidification – 4.00 degrees). For the esculin test, the observed changes in colour varied from yellow to dark brown. There were three replications of each experiment.

Cluster analysis (tree clustering) was applied to discover dissimilarities among fungal isolates according to their carbohydrate utilization profiles. Single linkage was used to determine the distances between clusters by the distance of the two closest objects (nearest neighbors) in the different clusters. Euclidean distance was chosen to compute.

Differences between carbohydrate utilization profiles of *B. bassiana* isolates were estimated by t-test for independent samples applying Descriptive statistics. Statistical analyses were performed using the software STATISTICA[R] version 6.0 of Stat Soft Inc.

Table 1. Origin of the isolates of the entomopathogenic fungus *B. bassiana*

Isolate of *B. bassiana*	Origin (initial host)	
	insect species	stage
433Bb	*Ips typographus* (Coleoptera: Curculionidae)	imago
434Bb	*I. typographus* (Coleoptera: Curculionidae)	imago
501Bb	*I. sexdentatus* (Coleoptera: Curculionidae)	imago
502Bb	*I. sexdentatus* (Coleoptera: Curculionidae)	imago
503Bb	*I. sexdentatus* (Coleoptera: Curculionidae)	imago
559Bb	*Dryocoetes autographus* (Coleoptera: Curculionidae)	imago
560Bb	*Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae)	larvae
561Bb	*Stenomax aeneus* (Coleoptera: Tenebrionidae)	imago
563Bb	*Hylurgops palliatus* (Coleoptera: Curculionidae)	imago
575Bb	*Lymantria dispar* (Lepidoptera: Lymantriidae)	larvae

RESULTS

Based on the acidification of their solutions, carbohydrates from the *B. bassiana* isolates were divided into four groups (Table 2). The first group (A) included carbohydrates that were utilized less than 10%, the second group (B) – between 10% and 50%, the third group (C) – between 50% and 70% and the fourth group (D) – greater than 70% of maximum acidification. We determined that the glycoside esculin and the aldopentose D-arabinose belonged to group A, as they were utilized only to a slight degree and only by four of the ten isolates examined. In those cases where esculin was the carbon source, the reaction was poor. It was at an average degree of 0.33 for three of the *B. bassiana* isolates, and at an average degree of 0.67 for one. D-arabinose was better utilized – from 0.67 to 2.00 (Table 2).

Table 2. Utilization of carbohydrates according to acidification of their solutions by *B. bassiana* isolates

Group	Carbohydrate	Acidification (average values in degrees) of carbohydrates by *B. bassiana* isolates									
		433Bb	434Bb	501Bb	502Bb	503BB	559Bb	560Bb	561Bb	563Bb	575Bb
A[1]	esculin	0	0	0.67	0.33	0.33	0.33	0	0	0	0
	D-arabinose	0	0	2.00	1.00	0.67	0	0	0	0	0.33
B[2]	dulcitol	1.33	1.00	0	3.00	3.00	0.67	0	0	0	0
	D-xylose	1.67	2.00	1.67	0.67	1.67	1.00	0	1.33	0	3.00
	raffinose	1.33	3.00	2.00	1.67	2.33	1.00	0	0.33	0	3.00
	galactose	2.00	1.33	4.00	1.33	0.33	4.00	0	2.00	0	0
	D-fructose	2.00	3.00	1.33	0.67	0.33	3.00	0.33	0.33	2.33	4.00
	adonitol	4.00	0.67	2.00	0	0	4.00	0.33	1.33	1.00	4.00
	rhamnose	1.67	2.67	1.00	3.67	2.67	3.00	0	0	0	3.00
	inositol	3.67	2.00	0	0.33	4.00	1.00	0	2.67	2.33	2.00
	lactose	3.00	3.00	1.00	3.00	2.33	2.67	0	0.33	0	3.33
C[3]	sorbitol	4.00	4.00	2.67	2.33	4.00	3.00	0.33	2.67	0	0
	dextrine	4.00	3.00	4.00	2.33	2.33	2.00	0	2.67	2.00	0
	mannitol	3.00	2.33	3.00	3.33	3.00	4.00	0	1.33	0.67	4.00
	salicin	4.00	4.00	1.33	2.00	2.33	3.00	0	4.00	0.33	4.00
	glycogen	4.00	4.00	2.67	4.00	1.33	4.00	0.33	4.00	0	2.00
D[4]	dextrose	3.67	3.00	2.67	3.67	3.33	4.00	1.00	4.00	3.00	1.33
	trehalose	4.00	3.67	3.33	2.67	2.67	4.00	2.00	2.67	2.67	4.00
	maltose	3.00	3.00	4.00	4.00	3.33	3.00	2.00	4.00	2.67	4.00
	sucrose	4.00	3.00	4.00	3.00	4.00	4.00	3.00	4.00	3.00	4.00

[1]group A – carbohydrates which utilized less than 10% of the maximum
[2]group B – carbohydrates which utilized between 10% and 50% of the maximum
[3]group C – carbohydrates which utilized between 50% and 70% of the maximum
[4]group D – carbohydrates which utilized greater than 70% of the maximum

Group B was the largest and it included dulcitol, D-xylose, raffinose, galactose, fructose, adonitol, rhamnose, inositol and lactose. We determined that the solution with dulcitol was acidified least. Dulcitol was not assimilated by five of the isolates tested. It was assimilated slightly by three isolates, and at an average degree of 3.00 by only two isolates. Lactose was utilized by eight isolates. Five of the eight were utilized at an average degree of over 2.67 (Table 2). However, lactose was not assimilated by the two isolates 560Bb and 563Bb.

Sorbitol, dextrin, mannitol, salicin and glycogen were included in group C. A positive reaction was recorded for almost all of *B. bassiana* isolates. Sorbitol was not assimilated by the two isolates – 563Bb and 575Bb, while dextrin was not assimilated by 560Bb and 575Bb, respectively. Salicin was not utilized by 560Bb nor was glycogen utilized by 563Bb.

The tested *B. bassiana* isolates exhibited a higher positive reaction (more than 70% of the maximum) to dextrose, trehalose, maltose and sucrose; consequently, these carbohydrates were included in group D. Dextrose was assimilated at the highest degree by 559Bb and 561Bb and with the exception of 560Bb and 575Bb (average degrees 1.00 and 1.33, respectively); all other isolates utilized the monosaccharide at an average degree over 2.67. *B. bassiana* isolates exhibited a higher level of enzyme activity for trehalase with the exception of isolate 560Bb which assimilated trehalose at an average degree of 2.00. The other four isolates varied from 2.67 to 4.00 (Table 2). Maltose and sucrose were the most split carbohydrates by all or most of the tested *B. bassiana* isolates.

According to the results of the cluster analysis of the carbohydrate utilization profiles, *B. bassiana* isolates could be divided into two larger clusters (Fig. 1).

The isolates 501Bb, 502Bb, 433Bb, 559Bb, 434Bb, 503Bb and 561Bb were included in one cluster, while isolates 575Bb, 560Bb and 563Bb were placed in the other.

Comparisons between profiles led to amalgamation of the more similar isolates into several small clusters. Linkage distance between isolates 433Bb and 559Bb formed a small cluster with a value of 4.71. The isolate 434Bb was linked to the cluster 433Bb – 559Bb in a new branch with a linkage distance of 4.73. The isolates 502Bb and 503Bb one by one amalgamated with the group. Both fungal iso-

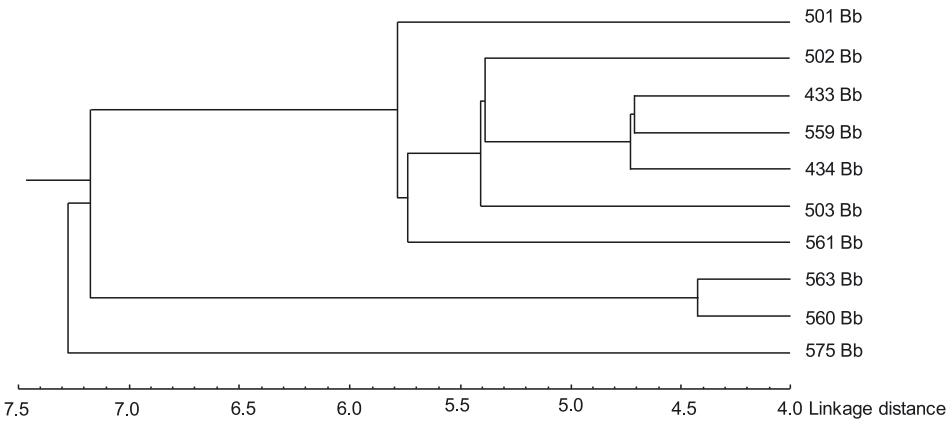

Fig. 1. Hierarchical tree diagram for *B. bassiana* isolates according to their carbohydrate accidification profiles

lates were obtained from the same initial host (the bark beetle *I. sexdentatus*) and they were more similar than different in their biochemical properties. Branches for the isolates 561Bb and 501Bb linked to the cluster developing the largest cluster in the hierarchical tree diagram with a linkage distance value of 5.78.

A cluster with the smallest dissimilarities included isolates 560Bb and 563Bb. Dissimilarities between them were expressed by a linkage distance value of 4.42 in the hierarchical tree diagram. This resulted from their different reaction to adonitol, glycogen, dextrin, dextrose, inositol, fructose, maltose, mannitol, salicin, sorbitol and trehalose.

The isolate 575Bb linking to the smallest cluster (isolates 560Bb and 563Bb) formed another distant cluster. The linkage distance between the isolate 575Bb and the objects in the largest cluster exhibited the highest value 7.27.

Based on their carbohydrate acidification patterns, comparison of the *B. bassiana* isolates were significantly different at p-level < 0.05. (Table 3).

Table 3. Isolates of *B. bassiana* significantly different according to their carbohydrate acidification patterns (t-test for independent samples)

Variables Isolates of *B. bassiana*	Mean group 1	Mean group 2	t-value	p
501Bb–560Bb	2.88	1.25	4.25	0.0001
502Bb–560Bb	2.5	1.25	3.20	0.0027
503Bb–560Bb	2.82	1.25	4.21	0.0001
433Bb–502Bb	3.52	2.5	3.03	0.0043
433Bb–503Bb	3.52	2.82	2.23	0.0315
433Bb–434Bb	3.52	2.85	3.18	0.0029
433Bb–563Bb	3.52	2.5	4.82	0.00002
433Bb–560Bb	3.52	1.25	7.94	0.0000
434Bb–560Bb	2.85	1.25	5.53	0.000003
559Bb–563Bb	3.25	2.5	2.73	0.0096
559Bb–560Bb	3.25	1.25	5.96	0.000001
561Bb–560Bb	2.90	1.25	4.44	0.000075
575Bb–560Bb	3.00	1.25	4.25	0.00013
563Bb–560Bb	2.50	1.25	4.30	0.000114

DISCUSSION

Our research determined that dextrose, trehalose, maltose and sucrose (group D) were the carbohydrates which were assimilated at the highest degree (more than 70%) by the *B. bassiana* isolates that we examined. Sorbitol, dextrin, mannitol, salicin and glycogen (group C) were utilized less (between 50 and 70%). Dulcitol, D-xylose, raffinose, galactose, fructose, adonitol, rhamnose, inositol and lactose (group B) were poorly assimilated (between 10 and 50%) and esculin and arabinose (group A) showed the poorest level of assimilation.

In general, carbohydrates from the last two groups (A and B) are not among ordinary substrates assimilated by entomopathogenic fungi. Most of them are widespread in different plant species: raffinose, rhamnose, fructose, inositol, esculin in the bark of the horse-chestnut; adoni-

tol in the plant *Adonis vernalis* as well as in the cell walls of Gram positive bacteria as a component of teichoic acids. Galactose, except in plants (in gums and mucilages) (Kretovich 1986; Lederkremer and Gallo-Rodriguez 2004) and dairy products, can be found as a part of glycolipids and glycoproteins in several human tissues. Dulcitol is a monosaccharide and a reduction product of galactose. Lactose is a disaccharide that is found most notably in milk. According to Lederkremer and Gallo-Rodriguez (2004), D-arabinose is not encountered frequently but is a constituent of important glycoconjugates and polysaccharides. D-xylose is present in widely abundant polysaccharides of plant tissues as a precursor to hemicellulose.

According to Campbell *et al*. (1983), L-rhamnose, lactose and arabinose were carbohydrates unsuitable for the growth of *B. bassiana* isolates.

Different results for acidification of esculin were determined by Rath *et al*. (1995) in experiments with isolates of *Metarhizium* spp. and *B. bassiana*, and by Todorova *et al*. (1998) who tested isolates of *Beauveria* and *Tolypocladium*. Esculin was metabolized by all the isolates that they examined.

We suggest that poor catabolism of the carbohydrates mentioned above might be due to the adaptation of fungal pathogens, in particular by *B. bassiana*, to insect hosts. Although *Beauveria* species do not possess species specificity, their host range does not include plant species. Studies on the establishment of *B. bassiana* as an endophyte in different plant seedlings did not determine the fungus as a phytopathogen (Posada and Vega 2005; Ownley *et al*. 2008; Vega *et al*. 2008). Their findings confirm the opinion of Evlakhova (1974) that *Beauveria* species are facultative saprophytes.

The adaptation to the entomopathogenic mode of life is a basic assumption for explaining the reduction of synthesis of some enzymes which become "unnecessary". Perhaps *B. bassiana* isolates obtained from the soil are richer in enzymes that make them more flexible. Thus, these isolates are able to survive better under soil conditions where they utilize carbon sources that do not serve as ordinary substrates that are assimilated by the entomopathogenic fungi. We suppose, that *B. bassiana* isolates established as endophytes are richer in enzymes opposite to isolates with a narrow host range.

The higher positive reaction exhibited by the carbohydrates included in groups C and D confirmed the presence of hydrolytic enzymes which are able to split these carbohydrates.

Previous studies (Campbell *et al*. 1983; Draganova and Trenchev 1993; Draganova 1997; Draganova and Lecheva 2002) showed that *B. bassiana* utilized mainly dextrose, mannose, sucrose, maltose and trehalose. According to Todorova *et al*. (1998), D-glucose (dextrose) and mannose were acidified by all strains of *Beauveria* spp. that were tested.

Catabolism of glycogen, and especially of trehalose, by the isolates we studied, correlates with the specialization of the fungus *B. bassiana* as an entomopathogen. Since trehalose is the main disaccharide found in insect hemolymph, glycogen is the main polysaccharide in insects, where it is stored in the fat body and in small amounts in the muscles.

According to Bidochka *et al*. (1990), the ability of *B. bassiana* to utilize trehalose indicates that it possesses trehalase. This might be an adaptation to nutrient availability in the insect hemolymph in which trehalose is a major soluble carbohydrate.

Results from cluster analysis of the carbohydrate utilization profiles of the *B. bassiana* isolates we studied, led to the formation of two larger clusters of isolates. The comparison between isolates in the largest cluster, according to their hosts, demonstrated that they were isolated from insects belonging to the Order Coleoptera – *S. aeneus* (Tenebrionidae), and the bark beetles *I. typographus*, *I. sexdentatus* and *D. autographus* (Curculionidae). Isolates from the other cluster were obtained from dead larvae of the Lepidoptera species *T. pityocampa* (Thaumetopoeidae) and *L. dispar* (Lymantriidae) and from an adult of the Coleoptera species *H. palliatus* (Curculionidae).

According to Becker (1988), not only the fungal species but also their forms and strains could be distinguished by their ability to assimilate carbohydrates, irrespective of rarely noted changes in their metabolism caused by environmental conditions.

We established in our research, that each *B. bassiana* isolate exhibited a different and specific carbohydrate utilization profile.

The most distinguishable profile was from the isolate 560Bb, which was isolated from the dead larva of *T. pityocampa* (Lepidoptera). Therefore, it was reasonable to expect dissimilarities in biochemical reactions with isolates obtained from Coleopteran hosts. Differences between the isolate 560Bb and the other nine isolates were highly significant at p-level < 0.005. Isolate 433Bb was significantly different from five of the studied isolates (Table 3) at p-level < 0.05.

The similarities among isolates suggest that these isolates belonged to the same species and that dissimilarities were related to individual biochemical characteristics.

Although our research included experiments with only ten isolates of *B. bassiana*, the results demonstrate that relations exist between carbohydrate profiles of fungal isolates and their original host. We conclude that *B. bassiana* isolates can be distingushed by their carbohydrate profiles.

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REFERENCES

Becker Z.E. 1988. Physiology and Biochemistry of Fungi. Moscow University Press, Moscow, 230 pp.

Bidochka M.J., Low N.H., Khachatourians G.G. 1990. Carbohydrate storage in the entomopathogenic fungus *Beauveria bassiana*. Appl. Environ. Microbiol. 56 (10): 3186–3190.

Bridge P.D., Abraham Y.J., Cornish M.C., Prior C., Moore D. 1990. The chemotaxonomy of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) isolates from the coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae). Mycopathologia 111 (2): 85–90.

Bridge P.D., Williams M.A.J., Prior C., Paterson R.R.M. 1993. Morphological, biochemical and molecular characteristics of *Metarhizium anisopliae* and *Metarhizium flavoviride*. J. Gen. Microbiol. 139 (6): 1163–1169. Doi: 10.1099/00221287-139-6-1163

Campbell R.K., Barnes G.L., Cartwright B.O., Eikenbary R.D. 1983. Growth and sporulation of *Beauveria bassiana* and *Metarhizium anisopliae* in a basal medium containing various carbohydrate sources. J. Invertebr. Pathol. 41 (1): 117–121.

Draganova S. 1997. Utilization of carbon sources by the *Beauveria* Vuill. genus (Moniliales, Deuteromycotina). Bulg. J. Agric. Sci. 3: 557–562.

Draganova S., Lecheva I. 2002. Discrimination between *Beauveria bassiana* strains isolated from *Agriopis bajaria* (Lepidoptera: Geometridae). Bulg. J. Agric Sci. 8: 43–48.

Draganova S., Trenchev G. 1993. A highly virulent strain of *Beauveria bassiana* (Bals.) Vuill. on the larvae of *Agrilus ribesi* Schaeff. (Coleoptera: Buprestidae). p. 241–245. In: Proc. the Second National Scientific Entomological Conference (G. Tsankov, ed.). PSSA, Sofia.

El-Sayed G.N., Ignoffo C.M., Leathers T.D., Gupta S.C. 1992. Use of a colorimetric system to detect enzymes expressed by germinating conidia of entomopathogenic fungi. Mycopathologia 118 (1): 29–36.

Evlakhova A.A. 1974. Entomogenous Fungi. Classification, Biology, Practical Significance. "Nauka" Press, Leningrad, 260 pp.

Kretovich V.L. 1986. Biochemistry of Plants. University Press, Moscow, 503 pp.

Lanyi B. 1987. Classical and rapid identification methods for medically important bacteria. Methods Microbiol. 19: 1–16.

Lederkremer R.M., Gallo-Rodriguez C. 2004. Naturally occurring monosaccharides. Properties and Synthesis. Adv. Carbohydrate Chem. Biochem. 5: 9–69.

Mugnai L., Bridge P.D., Evans H.C. 1989. A chemotaxonomic evaluation of the genus *Beauveria*. Mycol. Res. 92 (2): 199–209. Doi: 10.1016/S0953-7562(89)80012-7

Ownley B.H., Griffin M.R., Klingeman W.E., Gwinn K.D., Moulton J.K., Pereira R.M. 2008. *Beauveria bassiana*: Endophytic colonization and plant disease control. J. Invertebr. Pathol. 98 (3): 267–270. Doi: 10.1016/j.jip.2008.01.010

Pernfuss B., Schweigkofler W., Strasser H. 2003. Distinction of the entomopathogenic fungal species *Beauveria brongniartii* and *Beauveria bassiana* by comparing their carbon utilization patterns. In: „Entomopathogens and Insect Parasitic Nematodes: Current Research and Perspectives in Pest Biocontrol" (B. Papierok, ed.). IOBC/WPRS Bull. 26 (1): 121–123.

Posada F., Vega F.E. 2005. Establishment of the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte in cocoa seedlings (*Theobroma cacao*). Mycologia 97 (6): 1195–1200.

Rath A.C., Carr C.J., Graham B.R. 1995. Characterization of *Metarhizium anisopliae* strains by carbohydrate utilization (API 50 CH). J. Invertebr. Pathol. 65 (2): 152–161.

St. Leger R.J., Charnley A.K., Cooper R.M. 1986. Enzymatic characterization of entomopathogens with the APIZYM system. J. Invertebr. Pathol. 48 (3): 375–376.

Sun M.H., Liu X.Z. 2006. Carbon requirements of some nematophagous, entomopathogenic and mycoparasitic Hyphomycetes as fungal biocontrol agents. Mycopathologia 161 (5): 295–305. Doi: 10.1007/s11046-006-0249-9

Todorova S., Cote J.C., Coderre D. 1998. Distinction between *Beauveria* and *Tolypocladium* by carbohydrate utilization. Mycol. Res. 102 (1): 81–87.

Todorova S., Cote J.C., Martel P., Coderre D. 1994. Heterogeneity of two *Beauveria bassiana* strains revealed by biochemical tests, protein profiles and bioassays on *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) and *Coleomegilla maculate lengi* (Coleoptera: Coccinellidae) larvae. Entomophaga 39 (2): 159–169.

Vega F.E., Posada F., Aime M.C., Pava-Ripoll M., Infante F., Rehner S.A. 2008. Entomopathogenic fungal endophytes. Biol. Control 46 (1): 72–82. Doi: 10.1016/j.biocontrol.2008.01.008