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Rhynchites bacchus L. (Coleoptera: Rhynchitidae)'dan İzole Edilen *Beauveria bassiana*'nın Moleküler Karakterizasyonu ve Patojenitesi

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Özet

Yaprak kıvrım böceği (*Rhynchites bacchus* L., Coleoptera: Rhynchitidae) hemen hemen bütün Dünya'da elma, erik, kayısı, kiraz ve şeftali (nektarin) gibi pek çok meyvenin en önemli zararlılarından birisidir. Bu zararlı hem taş hem de yumuşak çekirdekli meyvelerle beslenir ve meyve bahçelerinde %80-90'a kadar zarara neden olabilirler. Bu çalışmada, daha etkili ve güvenli biyolojik mücadele etmeni bulmak açısından bu zararlının fungal patojenleri araştırılmıştır. Böcek örnekleri Türkiye'nin Gümüşhane ilinde 2007 yılının Temmuz ayında *R. bacchus* ile istila edilmiş elma bahçelerinden toplanmıştır. Bir fungal örnek izole edilmiş ve şu anda kullanılan morfolojik ve ITS, EF1-σ kısmi sekansını içeren moleküler teknikler kullanılarak karakterize edilmiştir. Bu tekniklere dayanarak, bu izolat *Beauveria bassiana* olarak tanımlanmıştır. Ayrıca bu izolatın *R. bacchus*'un erginleri üzerindeki insektisidal aktivitesi belirlenmiştir. Fungus 1×10^8 mL^{-1} spor konsantrasyonunun uygulanmasından sonra iki hafta sonunda %100 ölüme neden olmuştur ve bu fungusun LT50 değeri 7,5 gün olarak hesaplanmıştır. Bu çalışma *R. bacchus*'a karşı *B. bassiana*'nın etkinliğinin belirlenmesine yönelik ilk çalışmadır.

Anahtar Kelimeler: Yaprak kıvrım böceği, *Beauveria bassiana*, mikrobiyal mücadele

Molecular Characterization and Pathogenicity of *Beauveria bassiana* Isolated from *Rhynchites bacchus* L. (Coleoptera: Rhynchitidae)

Abstract

The leafroller weevil beetle (*Rhynchites bacchus* L., Coleoptera: Rhynchitidae) is one of the most destructive pest of various fruits such as apple, plum, apricot, cherry and peach (nectarine) etc. almost all over the world. This pest feeds either stone or pome fruits and can cause up to 80-90% damage to orchards. In this study, we investigated fungal pathogens of this pest to find more effective and safe biological control agent against it. Insect specimens were collected from apple orchards invaded by *R. baccus* during July of 2007 in Gümüşhane, Turkey. One fungal species was isolated and characterized by currently used morphological and molecular techniques including ITS and the partial EF1-α sequencing. Based on these characteristics, the isolate was identified as *Beauveria bassiana*. Separately, the insecticidal activity of the isolate was determined on *R. bacchus* adults. The fungus caused 100% mortality within two weeks days after application of 1×10^8 ml^{-1} spore concentration and the LT_{50} value of the fungus was calculated as 7.5 day. This is the first study to determine the effectiveness of *B. bassiana* against *R. baccus*.

Key words: The leafroller weevil beetle, *Beauveria bassiana*, microbial control

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1. Introduction

Rhynchites bacchus L. (Coleoptera: Rhynchitidae), the leaf roller weevil beetle, is one of the most important pests of plum, apple, and apricot as well as cherry and peach worldwide [1 and 2]. Adult beetles feed on buds, flowers and soft fruits. They lay eggs into a special chamber in fruit. Larvae feed mainly on decaying pulp of fruits, sometimes on seeds. In addition to its direct feeding damage, the fruits damaged by *R. bacchus* are often infected by moniliosis (*Sclerotinia fructigena*) which causes fruit to rot. The damage caused by this pest is very important and approximately 30- 40 pairs of adults per plum-tree can destroy the entire yield [2]. This species can cause very important economic loss in orchards compared to other *Ryhnchites* species since it nibbles fruit stalk in exceptional. In the northwest of Turkey, it was reported that it caused %80-90 harvest loss [2 and 3].

Burning-up of the plant residues in the gardens, picking dropped and mummified fruits from the infested trees and autumn plowing between tree rows are important preventive control measures against *R. baccus* [1]. Separately, some chemical substances such as carbayl 85%, methiocarb 50% and dioxacarbe 50% have also been utilized to control this pest [2 and 38]. However, these chemicals have undesirable side-effects to the environment, humans and plants. Therefore, scientists have been encouraged to search biopesticides such as insect pathogens to find more effective and safe biological control agents.

Entomopathogenic fungi are important natural enemies of insects and alternative to chemical insecticides because they are safer for the environment, humans and plants [5 and 6]. Additionally, entomopathogenic fungi are unique insect pathogens because they are able to infect their host via the external cuticle. Therefore, there is no need to be ingested to initiate infection with few exceptions such as *Ascosphaera*. This makes them primer candidates for use against plants sucking insects such as the many coleopteran insects, which have very few known viral or bacterial diseases [6-9]. Among many entomopathogenic fungi, much effort has been spent on the development of *B. bassiana* and *M. anisopliae* as biological control agents to be applied in both agriculture and forestry [10]. Up to now, the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin has been extensively used for the control of many important pests of various crops around the world and it has been tested on different target insects [11-20 and 40].

Although the leaf roller weevil beetle is a very important pest for many agricultural products all over the world, the control of this pest by fungal pathogens has been neglected. Since *R. bacchus* adults and larvae feeds within plant tissues such as buds, flowers and soft fruits, the use of entomopathogenic fungi for controlling this pest is promising. In the present study, we isolated and characterized *B. bassiana* isolate KTU-57 and tested its effectiveness on *R. bacchus* adults as a possible biocontrol agent. This is the first study to determine the effectiveness of *B. bassiana* against *R. bacchus*.

2. Materials and Methods

2.1. Collection of beetles

R. baccus larvae and adults were collected from the vicinity of Gümüşhane, Turkey in July of 2007. Insects were collected from the infested apple orchards and were placed individually into plastic

boxes (20 mm) with ventilated lids. When necessary, flying adults were collected by a trap and placed in the boxes (20 mm). Freshly collected apples were provided as food until they were transported to the laboratory. The collected insects were fed by apples in the laboratory and they were regularly checked with respect to fungal infection. A total of 215 beetles were inspected for the fungal infection.

2.2. Isolation of fungi

Fungi were isolated from the dead insects showing external fungal growth outside the insects. A small part of mycelia was taken from cadaver using an inoculation loop and was inoculated in the middle of Potato dextrose agar with 1% yeast extract (PDAY medium, Difco) including 50 µg/mL ampicillin and 50 µg/mL tetracycline (AppliChem) to prevent bacterial growth, and then plates were incubated at 28°C for two weeks. At the end of the growing period, the fungus was purified by single conidium and it was kept in a refrigerator at 4°C. Separately, the fungus was stocked in glycerol (15%) at -80°C.

2.3. Morphological characterization

Morphological identification of the isolate was performed according to the identification key prepared by Dr. Richard Humber [21]. The form of infection, the shape of colony and spores and the size of spores were initially used for morphological characterization. Microscopic preparations were prepared as described according to the identification key of Humber [21]. The fungus materials from 15-d culture were mounted in 50% acetic acid including aceto-orcein and were examined under a microscope.

2.4. DNA extraction and gene sequencing

The fungal isolate was derived from single conidia. To do this, conidial suspensions (1×10^7 ml^{-1}) from -80°C were plated on PDAY medium and incubated at 28°C for a week under 12:12 photoperiod. After that, a single colony was inoculated into 250 mL flask containing 100 mL Potato Dextrose Broth (PDB) (Merck, Germany). Liquid culture was shaken at 250 rpm at 28ºC for 1-2 weeks. After the incubation, cells were collected by filtering, frozen into liquid nitrogen, crushed with a mortar and 50 mg fungal biomass were used to extract DNA. Total genomic DNA was extracted using Nucleospin Plant kit (50 preps) according to the manufacturer's recommendations. Isolated DNAs were stored at -20ºC until use.

The ITS1-5.8S-ITS2 region (571 bp) of the nuclear rRNA-complex and the partial sequence of the elongation factor alpha-1 gene (EF1-α) (1.016 bp) were examined for further characterization of the fungus. Oligonucleotide primers, ITS4 (5'– TCCTCCGCTTATTGATATGC–3') as reverse primer and ITS5 (5'– GGAAGTAAAAGTCGTAACAAGG–3') as forward primer, were used for ITS PCR-amplification [22]. PCR amplifications were performed in a total volume of 50 µL, which included 5 µL 10X *Taq* DNA polymerase reaction buffer, 200 µM of each dNTPs, 50 pmol each of the opposing amplification primers, 2,5 unit *Taq*-DNA polymerase (Fermentase), and 50 ng genomic DNA. Thirty-five cycles were conducted in thermocycler (Eppendorf, mastercycler gradient, Hamburg, Germany): after the denaturation at 95ºC for 5 min, 95º C for 1 min, 55ºC for 55 s, 72ºC for 2 min, with a final extension at 72ºC for 10 min. An approximately 1.200 bp fragment of the EF1-α gene region was amplified using following primers: EF1T (5'-ATGGGTAAGGARGACAAGAC-3' and 1567R (5'-

ACHGTRCCRATACCACCSATCTT-3' as described by Rehner and Buckley 2005. PCR conditions were adapted essentially as described by Rehner and Buckley [23]. PCR products were separated on %1 agarose gel and visualized under UV light. Amplification products were extracted from agarose gels with the QIAquick Gel Extraction Kit (50 preps) and sent to MACROGEN (The Netherlands) for sequencing. Obtained sequences were used to carry out BLAST searches using the NCBI GenBank database to verify strain identification [24]. In addition, the sequences were used to compare the representative sequences from the study of Rehner et al. [25] (Table 1).

Table 1. Genbank sequences data of fungal isolates used in this study [25].

				GenBank number	
Species	**Strain**	**Location**	**Host**	**ITS**	***tef***
Beauveria amorpha	ARSEF 2641	Brazil	Hymenoptera: Formicidae	AY532008	AY531917
B. amorpha	ARSEF 4149	Australia	Coleoptera: Scarabaeidae	HQ880804	HQ881006
B. amorpha	ARSEF 7542	USA, Colorado	Hymenoptera: Formicidae	HQ880805	HQ881007
B. amorpha	B518ab	Chile	Soil	HQ880806	HQ881008
B. asiatica	ARSEF 4384	China	Coleoptera: Scarabaeidae	AY532026	AY531935
B. australis	ARSEF 4580	Australia	Orthoptera: Acridiidae	HQ880788	HQ880994
B. australis	ARSEF 4598	Australia	Soil	HQ880789	HQ880995
B. australis	ARSEF 4622	Australia	Orthoptera: Acridiidae	HQ880790	HQ880996
B. bassiana	ARSEF 300	Australia	Hemiptera: Lygaeidae	AY532015	AY531924
B. bassiana	ARSEF 751	Vietnam	Coleoptera: Chrysomelidae	AY532045	AY531954
B. bassiana	ARSEF 1040	Japan	Lepidoptera: Bombycidae	AY531972	AY531881
B. bassiana	ARSEF 1478	Brazil	Hemiptera: Pentatomidae	AY531981	AY531890
B. bassiana	ARSEF 1811	Morocco	Coleoptera: Curculionidae	AY531992	AY531901
B. bassiana	ARSEF 1848	Belgium	Coleoptera: Rhizophagidae	AY531995	AY531904
B. bassiana	ARSEF 7518	Japan	Hymenoptera: Pamphiliidae	HQ880762	HQ880975
B. brongniartii	ARSEF 617	France	Coleoptera: Scarabaeidae	HQ880782	HQ880991
B. brongniartii	ARSEF 979	France	Coleoptera: Scarabaeidae	HQ880783	HQ880992
B. brongniartii	ARSEF 985	Japan	Coleoptera: Scarabaeidae	HQ880768	HQ880978
B. brongniartii	ARSEF 2271	USA, Kentucky	Coleoptera: Curculionidae	HQ880779	HQ880988
B. brongniartii	ARSEF 2831	USA, Maryland	Lepidoptera	HQ880778	HQ880987
B. brongniartii	ARSEF 4362	Japan	Soil	AY532025	AY531934
B. brongniartii	ARSEF 4363	Japan	Soil	HQ880776	HQ880986
B. brongniartii	ARSEF 6213	USA, New York	Coleoptera: Curculionidae	HQ880775	HQ880985
B. brongniartii	ARSEF 6214	USA, New York	Coleoptera: Curculionidae	HQ880774	HQ880984
B. brongniartii	ARSEF 6215	USA, New York	Coleoptera: Curculionidae	HQ880781	HQ880990
B. brongniartii	ARSEF 7058	USA, Maine	Hymenoptera: Formicidae	HQ880773	HQ880983
B. brongniartii	ARSEF 7268	Republic of Korea	Coleoptera: Carabidae	HQ880772	HQ880982
B. brongniartii	ARSEF 7376	USA, Maryland	Homoptera: Cicadidae	HQ880770	HQ880980
B. brongniartii	ARSEF 7516	Japan	Coleoptera: Scarabidae	HQ880766	HQ880976
B. brongniartii	ARSEF 7517	Japan	Coleoptera: Scarabidae	HQ880767	HQ880977

B. brongniartii	ARSEF 10277	USA, Oregon	Soil: Rhizosphere	HQ880780	HQ880989
B. brongniartii	ARSEF 10278	USA, Oregon	Soil: Rhizosphere	HQ880769	HQ880979
B. brongniartii	ARSEF 10280	USA, Oregon	Soil: Rhizosphere	HQ880771	HQ880981
B. brongniartii	JE276c	Switzerland	Coleoptera: Scarabaeidae	HQ880784	HQ880993
B. caledonica	ARSEF 1567	Switzerland	Coleoptera: Scolytidae	AY531986	AY531894
B. caledonica	ARSEF 2251	Brazil	Coleoptera	AY532003	AY531912
B. caledonica	ARSEF 2567	Scotland	Soil	AY532006	AY531915
B. caledonica	ARSEF 4302	Australia	Soil	HQ880821	HQ881014
B. caledonica	ARSEF 7117	USA, Georgia	Orthoptera: Gryllacrididae	HQ880820	HQ881013
B. caledonica	ARSEF 8024	Denmark	Coleoptera: Scarabaeidae	HQ880818	HQ881012
B. kipukae	ARSEF 7032	USA, Hawaii	Homoptera: Delphacidae	HQ880803	HQ881005
B. malawiensis	ARSEF 7760	Malawi	Coleoptera: Cerambycidae	DQ376247	DQ376246
B. malawiensis	BCC17613d	Australia	NA	HQ880824	HQ881016
B. pseudobassiana	ARSEF 1855	Canada	Coleoptera: Scolytidae	HQ880796	HQ880999
B. pseudobassiana	ARSEF 2997	Canada	Hymenoptera: Vesidae	HQ880797	HQ881000
B. pseudobassiana	ARSEF 3216	USA, Wisconsin	Thysanoptera: Thripidae	AY532019	AY531927
B. pseudobassiana	ARSEF 3405	USA, Kentucky	Lepidoptera: Tortricidae	AY532022	AY531931
B. pseudobassiana	ARSEF 3529	USA, Maryland	Lepidoptera: Lymantriidae	HQ880795	HQ880998
B. pseudobassiana	ARSEF 4933	France	Coleoptera: Curculionidae	AY532029	AY531938
B. pseudobassiana	ARSEF 6229	China	Coleoptera: Scolytidae	HQ880799	HQ881001
B. pseudobassiana	ARSEF 7242	Republic of Korea	Hymenoptera	HQ880793	HQ880997
B. sungii	ARSEF 1685	Japan	Coleoptera: Scarabaeidae	AY531990	AY531899
B. sungii	ARSEF 5689	Republic of Korea	Coleoptera: Scarabaeidae	AY532030	AY531939
B. sungii	ARSEF 7043	Republic of Korea	Coleoptera: Scarabaeidae	AY532039	AY531948
B. sungii	ARSEF 7279	Republic of Korea	Coleoptera: Scarabaeidae	HQ880813	HQ881009
B. sungii	ARSEF 7280	Republic of Korea	Coleoptera: Scarabaeidae	HQ880814	HQ881010
B. sungii	ARSEF 7281	Republic of Korea	Coleoptera: Scarabaeidae	HQ880815	HQ881011
B. varroae	ARSEF 2694	Switzerland	Coleoptera: Curculionidae	HQ880802	HQ881004
B. varroae	ARSEF 8257	France	Acari: Varroidae	HQ880800	HQ881002
B. vermiconia	ARSEF 2922	Chile	Soil	AY532012	AY531920
Isaria cicadae	ARSEF 7260	Korea	Hymenoptera: Formicidae	HQ880826	HQ881017

2.5. Experimental Infection

100 µL spore suspension of the fungal isolate was plated on PDAY medium and incubated at 28°C for four weeks under 12:12 photoperiod. After growth period, conidia were harvested from by

adding 10 mL of sterile distilled water supplemented with 0.01% Tween 80 (Applichem, Germany). The conidial suspension was filtered through two layers of sterile muslin into 50 mL plastic tube (falcon) and then shaken for 5 min using a vortex. The concentrations of conidial suspension were adjusted to desired concentration using a Neubauer haemocytometer. The viability of the conidia of the isolate was tested by plating 100 µL conidial suspensions with the concentration of 1×10^6 conidia mL^{-1}. An examination of the culture after 24 h showed that about 95% of the conidia were viable.

R. bacchus adults were collected from naturally infested apple orchards in the vicinity of Gümüşhane, Turkey in August of 2007. They were transported to the laboratory and waited for 3 days so that they were acclimated to the laboratory conditions. After that, healthy adults were randomly selected and used in the bioassay. Ten adults for each replicate was applied to 1×10^8 mL^{-1} spore concentration by dipping into 15 mL of the conidial suspension and placed in a plastic box (20 mm) with a small apple as a diet. The control group was treated with 0.01% Tween 80 solution. Finally, all boxes were incubated at 28°C under 12:12 photoperiod and the mortalities of individuals were checked every 24 h. All experiments were repeated three times on different occasions.

2.6. Data analysis

Sequences were assembled and edited with BioEdit and aligned [26]. Cluster analyses of the sequences was performed using BioEdit (version 7.09) with Clustal W followed by p-distance method [27] with neighbor joining analysis using MEGA 5.0 phylogenetic software [28]. Alignment gaps were treated as missing data. Reliability of phylograms was tested by bootstrap analysis with 1000 replicates using MEGA.

Mortality data were corrected according to Abbott's formula [29]. The median lethal time (LT_{50}) was calculated based on Probit analysis using SPSS 15.0 statistical software.

2.7. Nucleotide sequence accessions number

The accession number of the sequences of ITS and EF1-α are FJ177448 and FJ177454, respectively.

3. Results and Discussion

There is an increasing request to search more reliable and efficient biological control agent against insect pests having agricultural and medical importance to reduce side effects of chemical insecticides and to provide ecologically acceptable pest control methods. Entomopathogenic fungi are an attractive, effective and environmentally safe alternative to chemical insecticides because they are safer for the environment, humans and plants. We collected *R. bacchus* adults from the vicinity of Gümüşhane, Turkey and investigated fungal infection of individuals to find more effective and safe fungal biocontrol agent against it. We were able to isolate only one isolate from adults which was covered with white mycelia resulting in 0.46% infection rate. Based on its morphological features, it was identified as *Beauveria bassiana*.

Additionally, we have sequenced ITS1-5.8S-ITS2 and the partial sequence of the EF1-α gene region for further characterization of the fungus. We used the BLAST search to perform DNA similarity

analysis of the isolate KTU-57. The isolate KTU-57 showed 100% similarity with different *B. bassiana* isolates based on ITS gene sequence (Table 2). Phylogenetic analysis also showed the isolate KTU-57 clustered with previously defined *B. bassiana* isolates according to the study of Rehner et al. [25] (Figure 1). Moreover, the isolate KTU-57 was shown to be similar to different *B. bassiana* isolates by 100 and 99% similarity index based on the partial sequence of EF1-α gene (Table 3). Also, this isolate was found to be similar to the representative *B. bassiana* strains from the study of Rehner et al. [25] (Figure 2). Based on all these results, it was concluded that the isolate KTU-57 is *B. bassiana*.

Table 2. Nucleotide sequence homology of ITS1-5.8S-ITS2 gene region belonging to *Beauveria bassiana* KTU-57 using the BLAST search.

Species	**Coverage (%)**	**ITS sequence similarity (%)**	**GenBank ID number**	**E value**
Beauveria bassiana	100%	100%	FJ177448	0.0
B. bassiana	100%	100%	FJ177450	0.0
B. bassiana	100%	100%	FJ177447	0.0
B. bassiana	100%	100%	FJ177446	0.0
B. bassiana	100%	100%	FJ177443	0.0
B. bassiana	100%	100%	FJ177441	0.0
Beauveria sp.	100%	99%	GQ354246	0.0
Cordyceps bassiana	100%	99%	AB079609	0.0

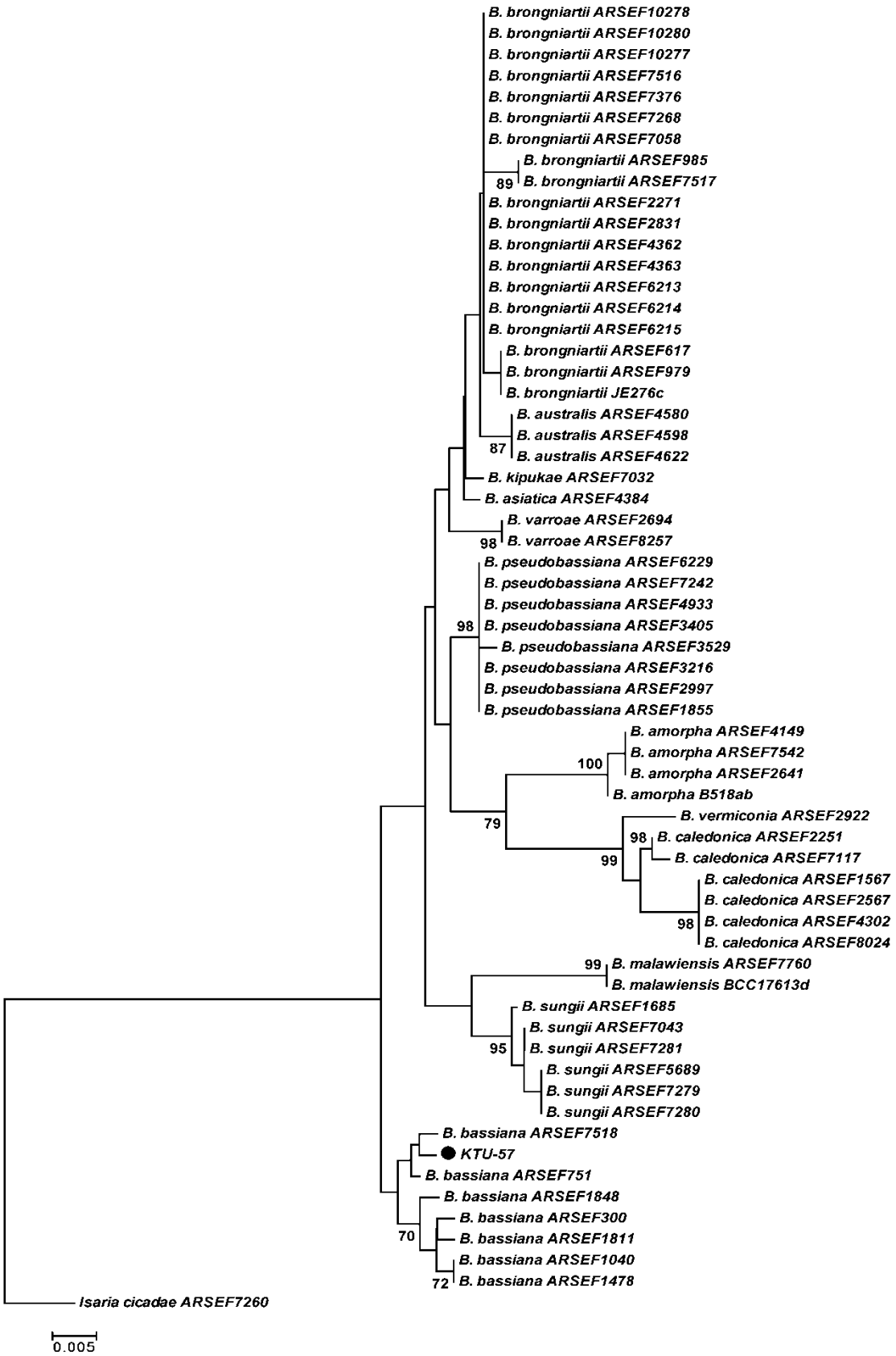

Figure 1. Pyhlogenetic position of the isolate KTU-57 within *Beauveria* genus based on ITS1-5.8S-ITS2 sequence. Representative strains were taken from the study of Rehner et al. [25]. Bootstrap value is shown next to nodes are based on 1000 replicates. The tree was rooted using isolate *Isaria cicadae* ARSEF7260 as the outgroup. Bootstrap values C≥70 are labeled. *Beauveria bassiana* KTU-57 was indicated with black dot. The scale on the bottom of the dendrogram indicates the degree of dissimilarity.

Table 3. Nucleotide sequence homology of the partial sequence of the EF1-α gene region belonging to *Beauveria bassiana* KTU-57 using the BLAST search.

Species	Coverage (%)	ITS sequence similarity (%)	GenBank ID number	E value
Beauveria bassiana	100%	100%	FJ177454	0.0
B. bassiana	100%	99%	EF193188	0.0
B. bassiana	100%	99%	EF193186	0.0
B. bassiana	100%	99%	AY883697	0.0
B. bassiana	100%	99%	AY883696	0.0
B. bassiana	100%	99%	AY531933	0.0
Cordyceps staphylinidicola	100%	98%	AY883701	0.0
C. bassiana	100%	98%	AY531971	0.0

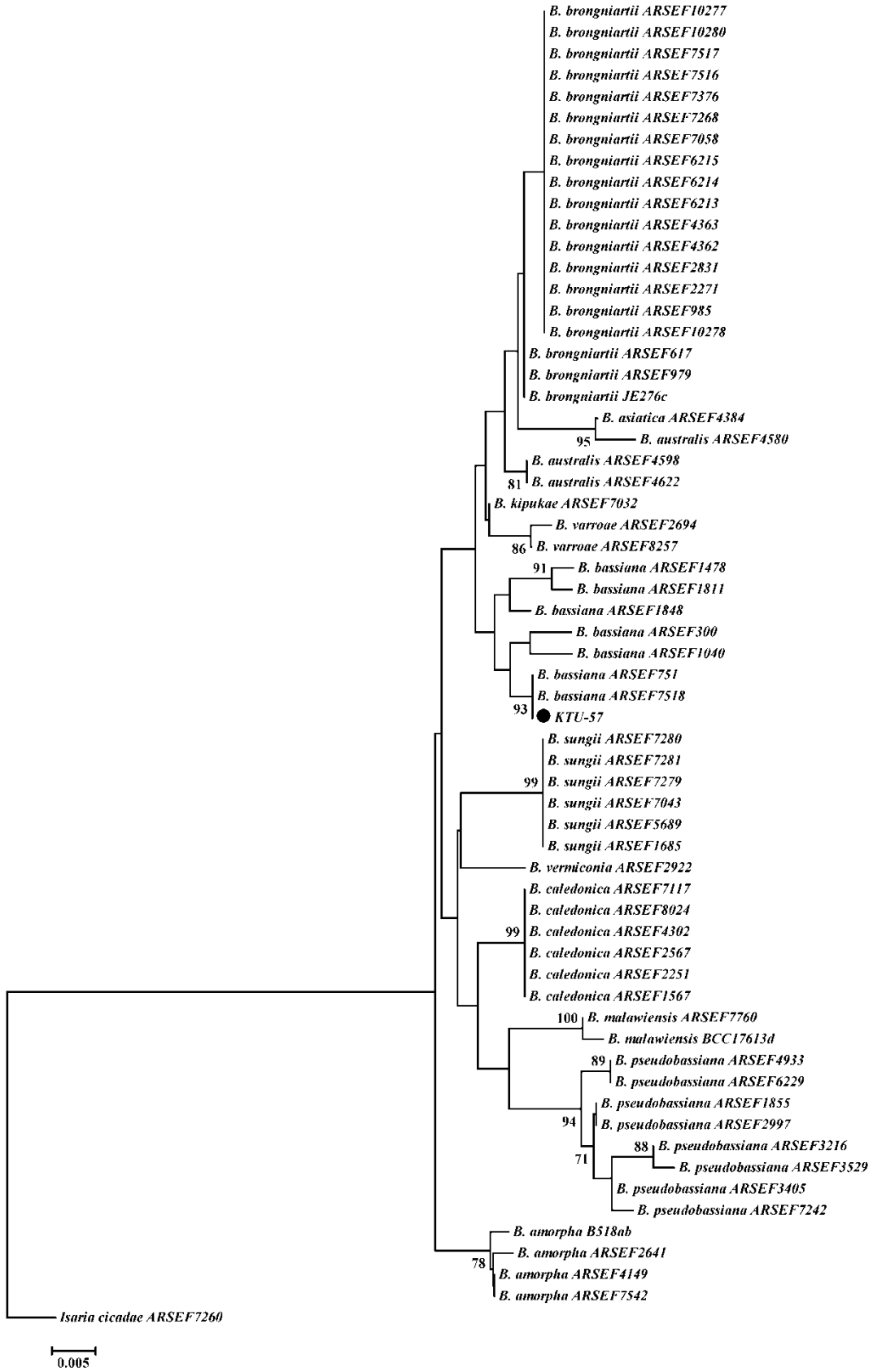

Figure 2. Pyhlogenetic position of the isolate KTU-57 within *Beauveria* genus based on the partial sequence of EF1-α. Representative strains were taken from the study of Rehner et al. [25]. Bootstrap value is shown next to nodes are based on 1000 replicates. The tree was rooted using isolate *Isaria cicadae* ARSEF7260 as the outgroup. Bootstrap values C≥70 are labeled.

Beauveria bassiana KTU-57 was indicated with black dot. The scale on the bottom of the dendrogram indicates the degree of dissimilarity.

The first step in the development and commercialization of microbial pest control products is to correctly identify candidate microorganisms that will be used against the target pest. The conventional identification studies are sometimes unsatisfactory to make conclusion on the identification of fungal species, within *Beauveria* genus in this case. Therefore, more detailed identification studies such as molecular characterization are required. In the present study, the identification of the isolate KTU-57 was supported by molecular characterization using two different gene sequences to clarify the precise taxonomic position of the isolate KTU-57.

We also tested the effectiveness of the isolate KTU-57 against *R. bacchus* adults under controlled laboratory conditions. It was shown that this isolate caused 100% mortality within two weeks after treatment of 1×10^8 mL^{-1} conidial concentration. The median lethal time (LT_{50}) was also calculated as 7.5 day (Figure 3). The most widely used species available commercially is *B. bassiana* and products based on this species are available for use against a very wide variety of insect pests, from *Cosmopolites sordidus* in Brazil [30] to pine caterpillars (*Dendrolimus* spp.) in China [31]. There are also many studies indicating the infection of different insect pests belonging to the family of Curculionidae with *B. bassiana* [11, 14, 32, 33 and 39]. This study also shows that the isolate KTU-57 seems to be a significant candidate against *R. bacchus* as a possible biocontrol agent.

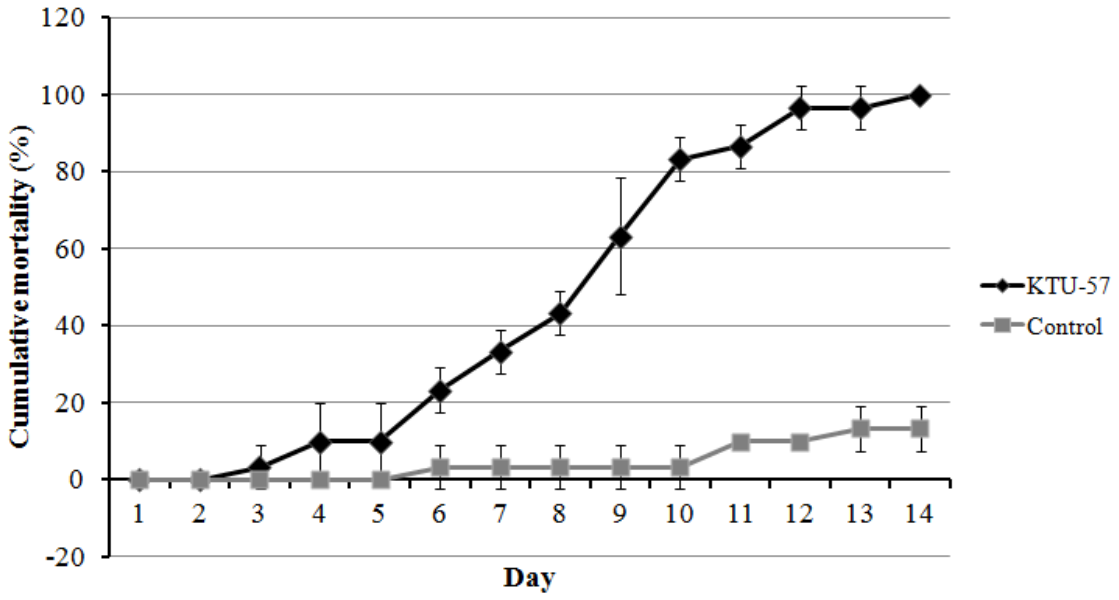

Figure 3. Cumulative mortality of *Rhynchites bacchus* adults after inoculation of 1×10^8 mL^{-1} conidia concentration of *Beauveria bassiana* KTU-57. The control group was treated with 0.01% Tween 80. Bar show standard deviation.

Investigation of the local fungal isolates against the target pest is always desirable because native isolates could have ecological compatibility with pest species, habitat type and geographical location [34-36]. Thus, native isolates will have a reduced risk of significant impact on environment and non-target insects in comparison to exotic isolates. The isolate KTU-57 is a native to Eastern Black Sea Region of Turkey, and therefore, it could be further investigated as a biological control agent against *R. bacchus*

since it might have some advantages in comparison to exotic isolates in terms of biological control. Additionally, the Eastern Black Sea Region of Turkey has favorable environmental conditions to use fungal entomopathogens in biocontrol programs because this region has a wet, humid climate and lower annual temperatures [4 and 37]. Entomopathogenic fungi require moisture for sporulation and germination of conidia; some even need high humidity to initiate infection. In addition, rain plays an important role in transmission of entomopathogenic fungi [6]. Based on all these information, *B. bassiana* KTU-57 seems to be promising candidate for controlling *R. bacchus*.

In conclusion, we isolated and characterized *Beauveria bassiana* KTU-57 from the leaf roller weevil beetle and determined its pathogenicity against *R. bacchus* adults. Our results suggest that the isolate KTU-57 appears to be good candidate for further investigation to control *R. bacchus* in the Eastern Black Sea Region of Turkey. Further studies are needed to determine the effectiveness of the isolate in the field. Additionally, the investigation of predisposition of the isolate KTU-57 to mass production should be also warranted.

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