A CONTRIBUTION TO MICROBIOLOGICAL CHARACTERIZATION OF BAT GUANO

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ABSTRACT: This study is based on the molecular and microbiological analysis of bat guano samples in an artificial gallery in Kırıkkale province. Guano samples were collected from the gallery floor into the falcon tube and kept at -20 °C in the laboratory. Guano samples were subjected to molecular analysis by applying dilution process. DNA isolation and rDNA sequence of *Bacillus cereus*, *Bacillus simplex* and *Kurthia gibsonii* from guano samples. This is the first study on the molecular identification of bacteria in guano piles in Turkey.

KEY WORDS: Bats, Guano, Bacteria, *Bacillus simplex, Bacillus cereus, Kurthia gibsonii,* Turkey

Up to date total of 38 bat species, one frugivorous and 37 insectivorous were recorded from Turkey (Albayrak, 2018). The bats live in all kinds of buildings, trees, mines, tunnels, rocks, wells and ruins. The bats have the nursery colonies formed only by the females and the winter colonies formed by the males and females for hibernation. Bat droppings, sea birds and seals manure piles accumulating in caves for hundreds of years are valued as guano, rich in basic elements and used as fertilizer in agricultural areas. In this regard, the bat guano is as effective as other bird guanos. Nearly all of the known caves in Turkey were screened and destroyed by people for guano.

Bats are the dominant animals of the cave ecosystem and accumulate droppings for thousands of years in their roosting places. Fertilizer microbiological reactions result in the increase of the elements such as nitrogen and the resulting guano becomes a productive mine. The research on the growth of five plant species, used bat guano, it was found that it was an hightly effective in growth (Sothearen et al., 2014).

In the bat guano samples from caves in Çorum, İstanbul, Kırklareli and Aydın provinces of Turkey were detected N, P, K, Ca, Mg, Cl, Na, and Fe elements (Altıntaş et al., 2005). Analysis carried out by Albayrak (2012) showed that N, P, K, Ca, Cd, Cu, Ni, Pb, Zn and Cr were found in the content of first manure of bat droppings accumulated in Havran artificial cave, Balıkesir province.

The objective of this study was to define some bacteria that play a role in the reduction or formation of guano.

MATERIALS AND METHODS

Guano samples were collected from a gallery at Keskin Vocational High School in the Kırıkkale Province. The gallery is completely dark used as a place for breeding of bats in summer (Fig. 1). Samples were taken using falcon tube. The strains were identified according to the methods described in “Bergey’s Manual of Systematic Bacteriology” (Claus & Berkeley, 1986) and on the basis of 16 s ribosomal DNA sequence. The stock culture was maintained on nutrient agar at 4

°C and as a glycerol stock at -20 °C. Later on 1 g of guano sample was serially diluted with sterile saline (0.9%). The diluted samples were then plated onto Muller Hinton agar plates. Plates were incubated at 37 °C for 24 h. These microorganisms have been purified.

Confirmation of the taxonomical status of the selected strains was done by 16S rRNA sequencing. Genomic DNA was isolated and analyzed from the isolates by the method of Tan & Yiap (2009). Bacterial 16S rDNA was amplified by using the universal bacterial 16S rRNA primers, 27F (5′-AGAGTT TGATCCTGGCTCAG-3′) and 1492R (5′-GGTGTTTGATTGTTACGACTT-3′) (Lane et al. 1985). PCR was performed with a 100 µL reaction mixture containing 10 µL (10 ng) of DNA extract as a template, 5 µL 16S Forward Primer (20 pmol), 5 µL 16S Reverse Primer (20 pmol) from each primer, 4 µL 50 mM $MgCl_2$ and 4 µL 5 mM dNTPs, as well as 1.5 U of *Taq* polymerase and buffer used. After the initial denaturation for 5 min at 94°C, there were 30 cycles consisting of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 5 min. PCR was carried out in a gene Thermal Cycler (Thermo Scientific, USA).

The PCR product was sequenced by 3730x1 DNA synthesizer (Applied Biosystems, USA). The two 16S rRNA sequences were aligned and compared with other 16S rRNA genes in the GenBank by using the NCBI basic local alignment search tools BLAST program (Benson et al. 2000). A distance matrix was generated using the Jukes-Cantor corrected distance model. The phylogenetic trees were created using Weighbor (Weighted Neighbor Joining: A Likelihood-Based Approach to Distance-Based Phylogeny Reconstruction).

RESULTS AND DISCUSSION

Guano samples were collected from a gallery at Keskin Vocational High School in the Kırıkkale Province. Bacterial isolates were coded as YA1, YB2 and YE3. Bacterial isolates were identified using the traditional morphological and biochemical tests. During this study, gram-positive, aerobic, non-motile, encapsulated rod bacteria were screened as presumptive *Bacillus* spp. (YA1 and YA2) and gram-positive, aerobic, non-motile, uncapsulated rod bacteria were screened as presumptive *Kurthia* spp. (YE3).

For the identification, 16S rRNA sequencing was used in our study. YA1 showed 99 % homology with *B. simplex* with an accession number of NJ115064, YB2 showed 99 % homology with *B. cereus* with an accession number of NR074540 and YE3 showed 99 % homology with *Kurthia gibsonii* with an accession number of NR025628, respectively (Fig. 2).

Bats live collectively and they accumulate fertilizers long years as some birds. Efficiency and sustainability in agricultural production are closely related to the physical, chemical and biological structure of the soil. The most common method used to correct and improve fungi, soil structure is the addition of organic matter to the soil. The most species of these ecosystems are bats.

Note: This study is a part of the Master Thesis of Yağmur Yük.

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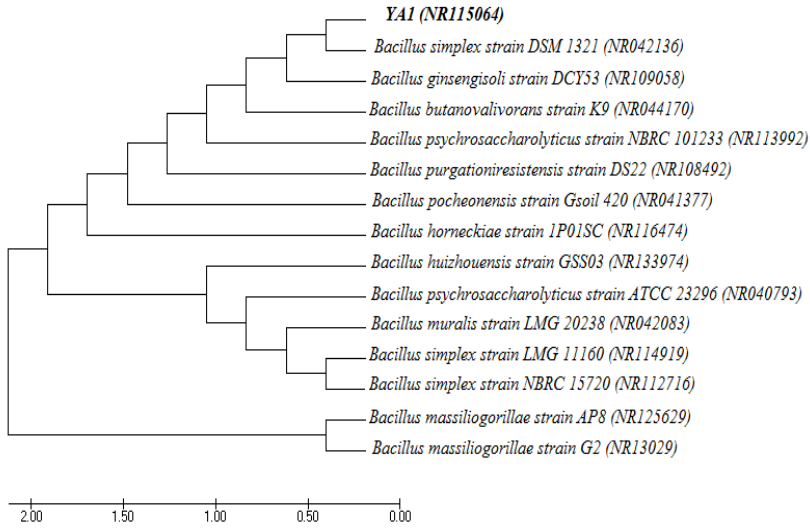
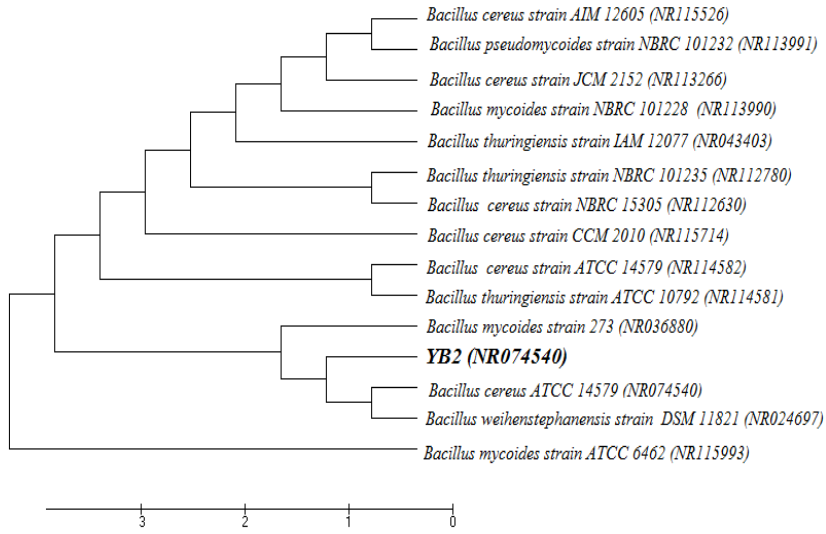
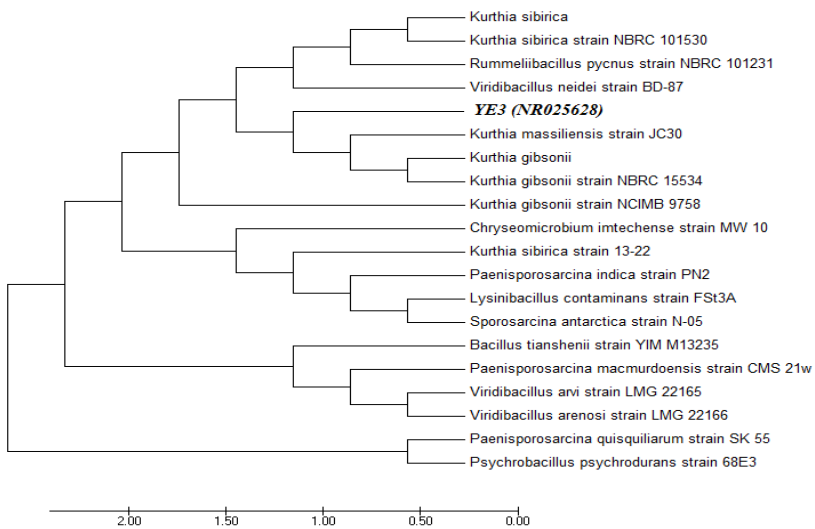
Figure 1. A small bat colony (*Myotis myotis* and *M. blythii*) in the gallery where the guano sample was taken (Photo: İ. Albayrak).



(a)



(b)



(c)

Figure 2. Phylogenic trees based on 16S rDNA gene sequence analyses of *B. simplex* (a), *B. cereus* (b), *Kurthia gibsonii* (c) isolates with an accession number of NJ115064, NR074540, NR025628 respectively. The 16S rDNA sequences were aligned and used to construct the neighbor-joining phylogenetic tree.