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Isolation of the Rhodotorula glutinis from the Mammillaria elongata Fruit Extract and Its Determination with 18S rRNA Gene Region

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ABSTRACT

The *Mammillaria elongata* is a species of the Cactaceae family, native to central Mexico. In Turkey, it is grown in the form of a cultivated plant. The succulent plants have pink and red fruits. In this study, we investigated the protein content, sugar content, the anti-bacterial and anti-fungal effect of the fruit extract. According to the results, a total wet weight of 0.252 g 15.5215 mg protein was found in the fruit with the Lowry method. Furthermore, the extract was found to contain high amounts of reducing sugar such as glucose. Bacterial growth (*Escherichia coli* and *Pseudomonas aeruginosa* on bloody agar) and fungal colonization (*Fusarium oxysporum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* on PDA) were not affected by the plant extract. However, in all of the control media which contained only the plant extract; a pink colored organism growth was observed. After DNA isolation and executing the 18S rRNA PCR analysis, the name of the species was determined to be the *Rhodotorula glutinis* according to % 100 sequence similarity.

Key words: *Mammillaria elongata*, *Rhodotorula glutinis*, 18S rRNA.

Mammillaria elongata Meyve Ekstraktından Rhodotorula glutinis'in İzolasyonu ve 18S rRNA Gen Bölgesi ile Belirlenmesi

ÖZ

Mammilaria elongata Cactaceae familyasına ait ve ana vatanı Meksika olan bir kaktüs türüdür. Türkiye'de ise kültür bitkisi olarak yetiştirilmektedir. Bu sukkulent bitkinin pembe ve kırmızı meyveleri bulunmaktadır. Bu çalışmada; bu bitkinin meyvelerinden elde edilen özütlerin şeker ve protein içerikleri, anti-bakteriyel ve anti-fungal etkileri araştırılmıştır. Sonuçlara göre; toplam yaş ağırlığı 0,252 g olan meyvelerde Lowry metodu ile 15,5215 mg protein bulunmaktadır. Bitki özütü yüksek miktarda glikoz gibi indirgen şeker içermektedir. Bitki özütü, kanlı agarda, *Esherichia coli* ve *Pseudomonas aeruginosa* üzerinde antibakteriyel; patates desktroz agarda (PDA) *Fusarium oxysporum, Rhizoctonia solani, Sclerotinia sclerotiorum* üzerinde antifungal bir etki göstermemiştir. Ancak, tüm kontrol besiyerlerinde pembe renkli bir organizmanın geliştiği gözlemlenmiştir. DNA izolasyonu ve 18S rRNA PCR'ı takiben bu organizmanın *Rhodotorula glutinis* ile % 100 sekans benzerliği gösterdiği belirlenmiştir.

Anahtar kelimeler: *Mammillaria elongata*, *Rhodotorula glutinis*, 18S rRNA.

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INTRODUCTION

The Mammillaria plants have been considered xerophytic plants that are resistant to dry weather and can conserve water in their parenchyma. *Mammillaria elongata* has been a cactus species with an erect or flat succulent stem. It is the most common variety of the cultivated plant. It usually contains pink or red fruits with high protein and sugar content (Bravo-Hollis 1978; Pilbeam 1999; Serrano & da Silva, 2008). This high protein and sugar ratio is preferred by endophytic microorganisms living in the plant.

The *Rhodotorula* species have been a yeast belonging to the Sporidiobolaceae family, which spreads in wide habitats. Yeasts with a colony morphology described as soft, moist and sometimes mucoid; contains red and pink pigments (Fell et al, 2000). Common in nature, it can be isolated from a variety of sources such as air, soil, seawater, plants, dairy products, and the home environment. Plants colonize humans and other mammals. The *Rhodotorula* species are known to be isolated from humans, plants and animals, as well as from foods such as fresh fruit juices, cheese, sausages, and some seafood. The *Rhodotorula* species produce the urease enzyme and do not ferment carbohydrates (Ahearn et al. 1962; Kutty and Philip 2008; Vishniac and Takashima 2010).

Some species are known to be pathogenic. It is observed that various systemic infections can develop in people exposed to the pathogen of *Rhodotorula*. They are defined as microorganisms commonly seen in hospital infections. In some cases, they have been isolated from the gastrointestinal tract. Because it is a ubiquitous saprophytic fungus and can be isolated from non-sterile human areas, it has been associated with infections such as meningitis, endophthalmitis, onychomycosis and prosthetic joint infections. It causes infection in the catheter, especially in people with hematological malignancies (Wirth & Goldani 2012).

Similarly, various infections in animals are caused by the *Rhodotorula* species. They have been the cause of some skin lesions in animals such as lung infections in sheep, dermatitis in cats and sea lions. Some studies have found these microorganisms in the ear canals of adult cattle, the oropharynx and cloaca of ostriches, the genitalia of monkeys, camels and cats in urban and rural areas. (Melville et al. 2004; Lord et al. 2010; Shrokri et al. 2010; Brotto et al. 2005; Costa et al. 2010).

In laboratory conditions, the *Rhodotorula* is not very picky; it grows rapidly in most media. The 18S rRNA regions can be exploited to molecularly detect the *Rhodotorula* species. In eukaryotic organisms, the 18S rRNA region is part of the 40S subunit. It is widely used as a biomarker because it is a protected area within the species and helps to conduct analysis at the species level (Wu et al. 2015). Although it cannot distinguish sufficiently at the species level, it is an important preferred marker for distinguishing between samples at higher taxonomic levels (Tang et al 2012), because it is evolutionarily conserved.

In this study, while the antibacterial and antifungal control of the extract obtained from the fruit extracts of *M. elongata* cacti was carried out, the process of obtaining the organism, which was obtained randomly and later determined to belong to the genus *Rhodotorula* according to 18 S rRNA analysis, is mentioned. Although the isolate was initially associated with external contamination, the same yeast obtained as a result of inoculation from the same plant extracts using the same method shows that the *Rhodotorula* yeast lives as an endophyte in the *Mammillaria* fruit.

MATERIALS AND METHOD

1. Extraction of the Mammillaria elongata fruits

Plant extraction has three stages; washing, drying and grinding. The *Red* fruits of the *M. elongata* should be washed with tap water and gently brushed to remove soil and other debris. After this process, the plant materials can be further washed with tap or distilled water. Drying was not performed in order to prevent loss of bioactivity of the plant material. Mechanical grinder should be employed to shred the plant tissues to various particle sizes. Ringer's lactate solution was used as a solvent during mechanical extraction. After grinding, the extract should be kept for storage from 3 to 7°C in the refrigerator until for the determination of their bioactivities. But extracts should not be stored in a deep freezer where precipitation may take place.

2. Determination of the protein and sugar content of the fruit extract

The Fruit extract contains a high amount of sugar and protein. The protein content was determined with the Lowry method (Lowry et al. 1951). The reduced sugar content was determined with the modified Lane Eynon methods (Hildreth & Brown 1942, Seoane et al. 2008).

3. The anti-bacterial and anti-fungal effect of the fruit extract

The antifungal and antibacterial activities of the plant extracts were investigated. *Esherichia coli* and *Pseudomonas aeruginosa* on the bloody agar (BA) media was used for antibacterial activity. And for antifungal activity; F*usarium oxysporum, Rhizoctonia solani* and *Sclerotinia sclerotiorum* on Potato dextrose agar (PDA) media was used. In aseptic conditions, after 100 microliters of plant extracts are added to each medium, *E. coli* and *P. aeruginosa* inoculated Blood Agar with the streak method (Weyland 1981). *F. oxysporum, R. solani* and *S. sclerotiorum* inoculated with the piece culture method. After inoculation, all cultures were incubated 28°C in the incubator for 7 days.

4. The isolation and purification of the *Rhodotorula* yeast from the control media

During the incubation period, pink colored, mucoid organism growth was detected in the control petri dishes. Control petries contained only 100 µl plant extract. This organism was purified from control petries from fresh media. The obtained pure organism was determined as the *Rhodotorula* yeast after morphological and microscopic investigation.

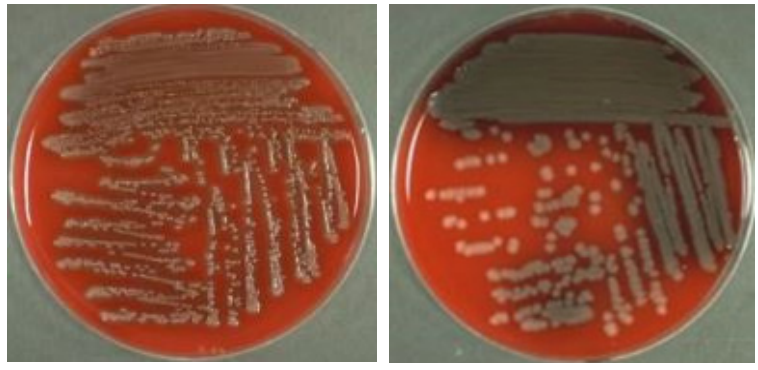
5. The DNA isolation, the 18S rRNA PCR and sequence analysis

The cetyltrimethyl ammonium bromide (CTAB) protocol was used for DNA isolation. The Quality of the DNA product was observed on 1% agarose gel electrophoresis and the amount of DNA was measured with the nanodrop spectrometer. The Universal 18S rRNA primers were used for PCR. The SILVA database was used for primer sequences (https://www.arb-silva.de/). GoTaq Hot Start Master Mix (Promega Corporation, USA) available from Promega was used for the polymerase chain reactions. In the preparation stage, 25 µl of Hot Start Master Mix, 1 µl Forward primer, 1 µl of Reverse primer, 2 µl of template DNA and 21 µl of nuclease-free water were used for 50 µl of the solution. The reaction conditions were carried out (pre-denaturation) at 95 ºC, annealing at 55 ºC (30 cycles) and extension 5 min at 72 ºC. The resulting PCR products were stored at -20 °C until use.

After the sequence analysis of the 18S rRNA gene region was completed, the resulting sequences were applied to the BlastN. According to BlastN results, the organisms showing highest sequence similarity were identified.

RESULTS AND DISCUSSION

The total protein amount of the extract which was obtained mechanically from *M. elongata* fruits with a total weight of 0.252 g each was found to be 15.5215 mg. This extract has a high content of reduced sugar. It is especially rich in glucose. The *Rhodotorula* produce urease and has the inability to assimilate inositol and to ferment sugars (Vazquez 2011). Some species of the *Rhodotorula* are the main carotenoid-producing microorganisms with predominant synthesis of β-carotene, torulene and torularhodin (Marova et al. 2012). Moreover, they are widely known as a good source of proteins, lipids and vitamins (Roadjnakamolson & Suntornsuk, 2010). Because of all these reasons, *Rhodotorula* spp. is important as commercially.



a b

Figure 1. a) Escherichia coli b) Pseudomonas aeruginosa on blood agar.

a b c

Figure 2. a) *Fusarium oxysporum* b) *Rhizictonia solani* c) *Sclerotinia sclerotiorum* on PDA.

In order to observe the antibacterial effect of the fruit extract, 100 µl extract was added to the blood agar and cultivated *E. coli* and *P. aeruginosa* under aseptic conditions (Fig 1a, 1b). Similarly, *F. oxysorum, R. solani* and *S. sclerotiorum* cultivated on PDA contain 100 ul fruit extract (Fig 2a, 2b, 2c). After the incubation process, there was no observation regarding any antibacterial and antifungal effect on cultivated petri dishes. However, especially on the all-control media, a new organism colonization which was pink colored and mucoid was observed (Fig 3a, 3b). According to microscopic observation, this organism may be considered as a kind of yeast since it was found to have a nucleus under X60 light microscopy (Fig 4). Interestingly, no traces of this organism were found in any of the cultivated petri dishes. It is considered that all bacteria and fungi which were used in this study, might utilize this organism as a nutrient.

A series of cultivations were made several times for the pure culture of the organism obtained from the control petri dishes. After pure culture, DNA extraction was made with the CTAB protocol. The amount of DNA was measured as 252 ng/µl and The A260/A280 value that gives the DNA purity was 1.87. Based on the sequence results from the 18 S rRNA PCR, the organism was identified as the *R. glutinis*. BlastN results showed that in the organisms, 18S rRNA sequence was similar to as %100 with the *R. glutinis* 18S rRNA gene region.

The *R. glutinis* has also been isolated from plants such as sugar beet and has been evaluated as an agent to prevent infection of the *R. solani* (El-Tarabily, 2014). However, in this study, this point is controversial since there is no inhibition in the development of the *R. solani*. Alongside this organism could be a contaminant or pathogen organisms which live in the *Mammillaria* fruit. Because, in some fruits, the *Rhodotorula* spp. is the cause of the infection and contamination (Heidenreich et al. 1997; Nagy et al. 2005). But biological replicates and aseptic conditions reduce this possibility.

a b

Figure 3. a) Contain only100 µl fruit extract petri dishes b) Colonization morphology of the purified organism.

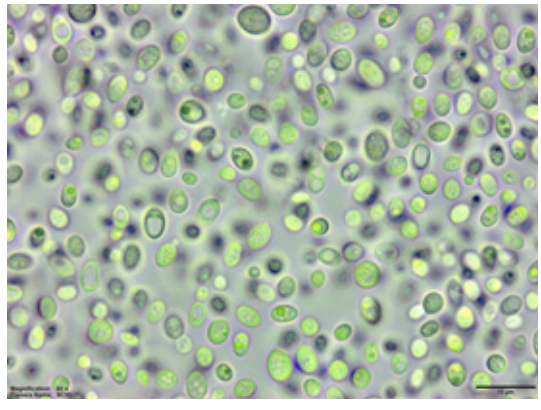


Figure 4. Microscopy Image X60.

AUTHOR CONTRIBUTION

Idea/Concept – FŞG, FGS; Design – FŞG, FGS; Consultancy – SA.; Data Collection and/or Processing –FŞG.; Analysis and/or Interpretation – FŞG, FGS.; Literature Review – FŞG Writing The Article -FŞG.; Critical Review – FŞG, FGS, SA.

CONFLICT OF INTEREST

All authors of this article declare that there is no conflict of interest. Also, we have no relevant financial interests in this manuscript.

FINANCIAL DISCLOSURE

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