BACTERIAL ANALYSIS OF MARKETED AND RAW HONEY IN TURKEY

Türkiye'de Marketlerden ve Üreticilerden Alınan Balların Bakteriyel Analizi

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Abstract: Marketed honey samples (the products of 15 different firms obtained from superstores), and raw honey (obtained directly from 11 different apiaries from the Black Sea and Marmara regions of Turkey) were analyzed for bacteria species presence. Out of the 26 honey samples, bacteria were isolated in 23. Twice the number of species was isolated from marketed as raw honey. However, neither European Foulbrood (EFB) (*Melissococcus pluton*) nor American Foulbrood (AFB) (*Paenibacillus larvae larvae*) was detected in any of the samples. This suggests that sanitary measures and disinfection requirements may not be met in collecting, packaging and labeling honey for marketing.

Key words: Honey, *Bacteria,* American Foulbrood, European Foulbrood.

Özet: Ülkemizde marketlerden (15 adet firma ve 11 adet arıcı) ve Marmara ve Karadeniz Bölgesi'nde doğrudan arıcılardan toplanan 26 ham bal numunesi bakteri varlığı bakımından analiz edilmiştir. Toplam 26 numune örneğinden 23'ünde bakteri varlığı tespit edilmiştir. Marketlerden toplanan ballardan ham bala göre iki kat daha fazla bakteri türü izole edilmiştir. Numunelerin hiç birinde Avrupa Yavru Çürüklüğü (EFB) etkeni (*Melissococcus pluton*) ve Amerikan Yavru Çürüklüğü etkeni (AFB) (*Paenibacillus larvae larvae*) bulunamamıştır. Bu çalışmanın sonuçları dezenfeksiyon ve temizlik ölçütlerinin toplama, paketleme ve etiketleme sırasında yeterince yapılmadığını göstermektedir.

Anahtar Sözcükler: Bal, Bakteri, Amerikan Yavru Çürüklüğü, Avrupa Yavru Çürüklüğü.

INTRODUCTION

Honey is used as a medicine in many cultures around the world (see reviews: Kaal 1991, Graham 1992, Molan 1992, Molan 1999, Doğaroğlu 1999, Molan 2001, Kumova and Korkmaz 2001). Although honey has anti-microbial activity due to its osmotic effect and chemical constituents, there are microorganisms that either have resistant spores or can remain dormant in honey. Among the most damaging to beekeeping are *Paenibacillus larvae larvae*, which cause American Foulbrood (AFB), and *Melissococcus pluton*, which causes European Foulbrood (EFB) (Morse and Nowogrodzki 1990, Bailey and Ball 1991).

AFB spores are incredibly resistant to environmental factors, can survive for years (over 35), and are not readily destroyed even in boiling water (Hornitzky 1998). Although adult bees are resistant to AFB, spores may be transmitted to larvae by adult bees (Bailey and Ball 1991, Hansen and Brodsgaard 1999). Honeybee colonies may contain honey with large numbers of spores and not show clinical signs, at which time the disease is still very transmissible to other colonies by frame movement among hives or into an apiary by swarm capture (Morse and Nowogrodzki 1990, Bailey and Ball 1991, Hornitzky 1998, Hornitzy et al. 1996).

EFB is seasonal in nature, and although not as serious a disease as AFB, it still causes widespread

colony losses in Europe as well as other parts of the world (Morse and Nowogrodzki 1990, Bailey and Ball 1991). Like AFB, EFB spores may be detected in honey one year before any clinical symptoms are noticed. But unlike AFB, the disease is characterized by the presence of the secondary invading microorganisms such as *Paenibacillus alvei (Bacillus alvei), B eurodise, B lateresporus*, and *Enterococcus faecalis* (Djordjevic et al. 1998, Hornitzky and Smith 1998, Spivak and Gilliam 1998, Spivak and Gilliam 1998).

With trade restrictions being relaxed as Europe becomes a free trade community, early detection of these diseases becomes paramount for a healthy European beekeeping industry (Martin 2002, Mutinelle et al. 2002). Turkey represents a frontline in that effort because it is a conduit to Europe from both the Middle East and Central Asia due to its geographical location, and because it has a large non-regulated migratory beekeeping industry. Although both AFB and EFB are known in Turkey by beekeepers, only two local studies of the incidence of theses disease have been performed: 1) 10% of the marketed and 14% of the raw honey samples contained *P larvae* in Ankara province (Aydın et al. 1999) 2) *P larvae, M pluton, P alvei,* and *E faecalis* were found in some brood frames taken from colonies in Ankara province (Özkırım and Keskin 2002). In this study, we examined marketed and raw honey from the two migratory beekeeping centers in Turkey to determine the bacteria present and stages of contamination. This represents the first widespread study of this type in Turkey, and we hope that it will be a significant step towards regional honeybee disease control.

MATERIALS AND METHODS

Honey samples were randomly chosen from 15 superstores (the products of 15 different firms=marketed honey) and 11 apiaries (raw honey). Stores and apiaries were located across the Marmara and Black Sea regions of Turkey, which represents an area that stretches from Greece to the Republic of Georgia. These regions have the highest number of apiaries in Turkey and represent the bulk of the migratory beekeepers in the country. Honey samples were kept at room temperature for a few days until analysis.

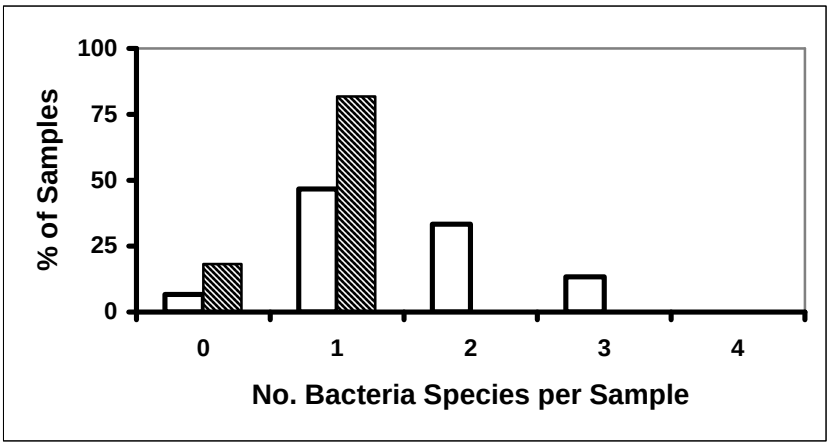
Honey samples were individually analyzed using the following protocol: 1ml of honey was homogenized with Tripticase Soy Broth (BBL-BD, Cockeyville, USA) and then transferred to enriched BACTEC PLUS medium and placed in Aerobic/F bottles in a BACTEC 9240 automated system (BD, Sparks MD, USA) for up to 7 days. After the growth signal was observed, samples were transferred to 5% Sheep Blood Agar, SBA (Bio-Morieux, France) and incubated at 37ºC for 24-48 hours. Cultured bacteria were gram stained, and morphological assessments were made microscopically. Bacteria of different morphologies were further characterized with BBL CRYSTAL kits (BD, Aalst, Belgium) to obtain species identification. This protocol has been reported as an accurate, reliable tool for identification of gram positive and negative microorganisms, including *P larvae, M pluton* and secondary invaders (Dobbelaere et al. 2001, Chantawannakul and Dancer 2001).

RESULTS

Bacteria were isolated from 23 of the 26 samples (88.5%). From the raw honey, 5 bacteria species were identified: *Bacillus brevis, B cereus, B licheniformis, B subtilis*, and *Corynebacterium aquaticum.* In addition to those five species, an additional 5 were isolated from the marketed honey samples: *Bacillus sphericus, Paenibacillus alvei, Staphylococcus aureus, Streptococcus anginosus* and *S. vestibularis*. All bacteria species found in the raw honey samples were also found in the marketed honey. Neither AFB nor EFB were isolated from any of the samples. However, the secondary invader *P. alvei* that is associated with EFB was isolated from one sample (marketed).

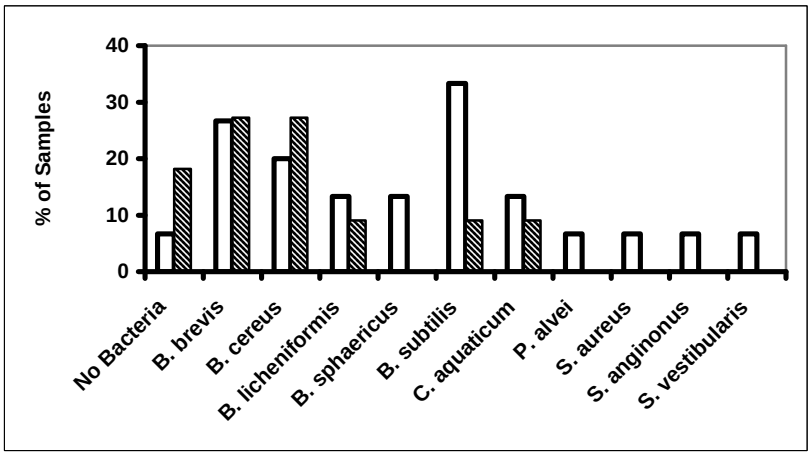
The average number of bacteria species per market sample was significantly greater ($t=2.52$, $df=24$, $P<0.05$) than the mean for the raw honey (Fig. 1).

Figure 1: Number of bacteria species per sample versus percentage of the samples. Hatched bars are raw honey and white bars market honey data. Marketed honey is characterized by generally having more bacteria species per sample.



Also, with the exception of *B brevis*, the percent of samples with each bacteria species was greater in the marketed honey (Fig. 2). Neither market nor raw honey samples (number of bacteria species per sample) were Poisson distributed (Coefficient of Dispersion: market=0.46, raw=0.20).

Figure 2: Bacteria species versus percentage of the samples with that microbe. Hatched bars are raw honey and white bars market honey data. Notice that more species were isolated from market honey, and that marketed honey was more likely to have each species with the exception of *B brevis* and *B cereus.*



DISCUSSION

The isolated *Bacillus* species and *C aquaticum* are widespread in nature (Funke and Bernard 1999, Loga and Turnbull 1999). Therefore, their presence in comb and honey are unavoidable simply from the activities of the bees themselves. However, the differences between the marketed and raw honey samples should be noted. Since a greater diversity of bacteria was found in marketed honey, mixing honey when shipping and packaging cannot account for the differences observed. This suggests that beekeeping equipment and processing procedures are responsible for some of the microflora found. The departures from frequencies expected by a Poisson process also suggest that honey is systematically exposed to bacteria, first by bees in the hive and subsequently by people packaging honey.

Microorganisms do not grow well in natural honey, and artificial honey and sugar solution do not show the same antibacterial effect of natural honey (for review: Molan 1992). One of the bacterium, *S*

aureus, found in one of the market samples is actually very susceptible to the antibacterial aspects of honey, which suggests packaging of uncured honey or addition of artificial honey in this case (Molan 1992).

Even though Aydın *et a*l. (1999) isolated *P larvae* in marketed and raw honey samples, and Ozkırım & Keskin (2002) reported that they isolated *P larvae* and *M pluton*, we did not (exact test P=0.02 of obtaining this result by chance alone). However, we did find the EFB secondary invader *P alvei* in a honey sample. Further, *B cereus* and/or *B subtilis* were present in 50% of the samples, and those species have also been defined by some to be EFB secondary invaders (Zeybek H. 1991).

Schuch *et al*. (2001) reported an improved method for the detection of *P larvae* in honey using PLA medium. They examined 137 imported honey samples and found 24 spores on PLA and no spores on Thiamine-brain heart infusion agar, J-agar, or Bailey and Lee agar. This result suggests that our finding of no *P larvae* in Turkish honey does not mean those honey samples were free of spores even though both AFB and EFB have been successfully isolated using the methods we employed (Hornitzky and Smith 1998, Funke and Bernard 1999), but probably are in very low concentrations if present. However, *Bacillus* and other genera we isolated here might adversely affect the systems ability to isolate *P larvae*.

In summary, beekeepers and honey firms must pay more attention to sanitary measures when collecting and packaging honey. Those sanitary measures will undoubtedly be reinforced by new EU regulations on bee products. The widespread occurrence of species associated with *M pluton* suggests that EFB may be a major problem in the near future.

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