A Research Note

Effect of Honey on Bacterial Growth and Spore Germination

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ABSTRACT

The effect of honey on the growth of Bacillus cereus, Staphylococcus aureus, Salmonella dublin and Shigella dysenteriae was examined in this study. Gram-positive bacteria were found to be less sensitive to the inhibitory action of honey compared to gram-negative bacteria. Significant growth inhibition (p<0.05) of S. dublin and S. dysenteriae was obtained by using 2% (vol/vol) honey. Spore germination of B. cereus was also significantly inhibited (p<0.05) by 2% (vol/vol) honey. The inhibitory factor(s) is under investigation.

Key Words: Honey, inhibition, spores, Bacillus, Staphylococcus, Salmonella, Shigella.

The use of honey in the treatment of wounds has been reported in several instances (4,6,7). The antibacterial activity of honey has been attributed to its high osmolarity (2,6), hydrogen peroxide production (1) and to an unknown factor(s) found in certain types of honey such as Manuka honey (9). The sensitivity of wound infecting bacteria to different types of honey was reported (9). Honey extract was found to inhibit basophils degranulation induced by anti-immunoglobulin E (anti-IgE) antibody (8). The aim of this study is to determine the effect of honey on the growth of certain types of bacteria known to cause gastroenteritis.

MATERIALS AND METHODS

Bacillus cereus American Type Culture Collection (ATCC) 11778, S. aureus ATCC 6530, S. dublin A 4950/80 and S. dysenteriae were used in this study. They were grown and maintained in Brain Heart Infusion (BHI) medium. An inoculum of each bacterium was prepared by growing it overnight in a rotatory shaker incubator at 37°C in 100 ml portions of BHI broth. Cells were centrifuged and resuspended in a small volume of fresh medium. This suspension was used to inoculate 100 ml BHI broth containing 1 or 2% (vol/vol) honey (Langnese, HGC, P 11/92, Germany). After 3, 5, 8, 24, 48 and 72 h incubation at 37°C, samples were withdrawn to measure absorbance at 420 nm using Baush & Lomb spectronic 21 spectrophotometer. The inhibition was calculated by comparing the overall absorbance readings for 1 and 2% effects to the readings of 0% at the incubation intervals.

To measure leakage of intracellular material, a 10 ml culture grown in 2% concentration of honey was withdrawn after 24 h incubation and centrifuged at 1,000 x g for 10 min. Supernatants were read for their absorbance at 260 nm.

Spore germination of B. cereus was determined by centrifugation of about 100 ml culture of B. cereus in its log growth phase at 2,500 x g for 15 min, after which the pellet was resuspended in 5 ml penassay broth (Difco Laboratories, Detroit, MI), inoculated in 500 ml penassay broth and incubated overnight at 37°C with vigorous aeration. Cells were then centrifuged at 2,500 x g for 15 min and resuspended in 30 ml of 0.1 M potassium hydrogen phosphate (KH₂PO₄) 0.01 M MgCl₂ solution (pH 7.8) containing 500 µg/ml lysozyme. After incubation at 37°C for 1 h, the released spores were centrifuged then resuspended in 30 ml sterile distilled water and heated in a water bath at 80°C for 30 min. The spore suspension was re-centrifuged and resuspended in 20 ml sterile distilled water. Then 1 ml of B. cereus spores were inoculated, in duplicate, into penassay broth containing 1 and 2% (vol/vol) honey, and the broth was incubated in shaker water bath at 30°C. The germination of B. cereus spores was studied at 3, 5, 8, 24 and 48 h incubation by monitoring the extinction at 600 nm according to Coote (3). All techniques used in this study were conducted in duplicate.

Analysis of variance was carried out on the obtained data using the Tukey-Kramer test in the STAS module of SYSTAT. This was to figure the mean level differences of 1 and 2% of honey concentrations on the tested bacterial species at 0 to 8 h and at 24 to 72 h incubation times using 0% honey concentration as control group. All statistical analysis was performed using SYSTAT (10).

RESULTS AND DISCUSSION

The bacterial species used in this study are known for their role in food poisoning (5). Table 1 shows that no significant inhibition (p>0.05) of the bacteria could be detected within 0 to 8 h of incubation. By comparing the readings recorded along the whole incubation time for 1% and for 2% honey to the readings for 0%, the absorbance of B. cereus was diminished 16.3 and 20.3%, respectively, after 24 to 72 h. However, this inhibitory effect was statistically not significant (p=0.08). Significant differences (p<0.05) among absorbencies of S. aureus could be detected after 24 to 72 h. By using 2% (vol/vol) honey, 12
TABLE 1. Effect of honey on growth of some pathogenic bacteria.

<table>
<thead>
<tr>
<th>Bacteria spp.</th>
<th>Time/h</th>
<th>0%</th>
<th>1%</th>
<th>2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>0 to 8</td>
<td>0.080 + 0.100</td>
<td>0.106 + 0.107</td>
<td>0.092 + 0.099</td>
</tr>
<tr>
<td></td>
<td>24 to 72**</td>
<td>1.770 + 0.175</td>
<td>1.616 + 0.129</td>
<td>1.350 + 0.059</td>
</tr>
<tr>
<td>B. cereus</td>
<td>0 to 8</td>
<td>0.332 + 0.438</td>
<td>0.347 + 0.439</td>
<td>0.362 + 0.439</td>
</tr>
<tr>
<td></td>
<td>24 to 72</td>
<td>1.592 + 0.351</td>
<td>1.240 + 0.091</td>
<td>1.137 + 0.064</td>
</tr>
<tr>
<td>S. dublin</td>
<td>0 to 8</td>
<td>0.467 + 0.412</td>
<td>0.454 + 0.377</td>
<td>0.439 + 0.373</td>
</tr>
<tr>
<td></td>
<td>24 to 72**</td>
<td>1.695 + 0.208</td>
<td>1.089 + 0.088</td>
<td>0.962 + 0.072</td>
</tr>
<tr>
<td>S. dysenteriae</td>
<td>0 to 8</td>
<td>0.342 + 0.353</td>
<td>0.338 + 0.361</td>
<td>0.360 + 0.330</td>
</tr>
<tr>
<td></td>
<td>24 to 72**</td>
<td>1.755 + 0.362</td>
<td>0.898 + 0.119</td>
<td>0.827 + 0.116</td>
</tr>
</tbody>
</table>

* Mean ± Standard deviation.
** Significant difference between the concentrations (p<0.05).

and 21.5% significant (p<0.05) inhibition of S. aureus had occurred after 24 and 72 h, respectively.

In 2% (vol/vol) honey, the growth of S. dublin and S. dysenteriae were inhibited 21.3 and 22%, respectively, after 24 h while after 72 h incubation they were significantly inhibited 33.2% (p=0.001) and 40.8% (p=0.005), respectively. Gram-negative bacteria were more sensitive to the inhibitory action of honey than gram-positive bacteria. The S. dysenteriae was found to be the most sensitive among the tested bacteria to the effect of honey.

Honey significantly (p<0.05) inhibited germination of B. cereus spores (Fig. 1). One percent and 2% concentrations inhibited germination 37% (p=0.03) and 53.3% (p=0.009), respectively, within 24 h while after 72 h these two concentrations inhibited the spore germination 34% (p=0.03) and 64.8% (p=0.028), respectively. This indicates that 2% honey concentration is more effective against B. cereus spores by prolonged incubation. Although high osmolarity is one of the factors attributed to the effect of honey on bacteria, the use of 1 and 2% (vol/vol) honey excludes the effect of high osmolarity (2,6).

Figure 1. Effect of honey on germination of spores of B. cereus. Columns: White: 0%; Dotted: 1%; Black: 2% honey concentration.

Two percent honey caused a significant (P=0.015) leakage of intracellular material for all tested bacteria after 72 h incubation (Fig. 2). This suggests that certain factors present in honey have an effect on the bacterial cell wall, possibly leading to the lyses of the bacteria. Possible identification of such inhibitory compound(s) is in progress. Different types of honey exert different degrees of inhibition on different species of bacteria (9). New Zealand Manuka honey exerted 100% inhibition of S. aureus using 1.8% (vol/vol). However, S. aureus was found least sensitive to other types of New Zealand honey (9). In conclusion, honey was found to inhibit the growth of bacteria and the spore germination of B. cereus.

REFERENCES


preparation. Although the remaining toxin levels found in the meal are low if we consider the LD$_{50}$ required to elicit toxicity in rats and poultry (4, 5), toxin contamination in the meal could be important because of possible synergism among *Alternaria* metabolites and the presence of other minor toxins such as alternatoxins, whose toxicity and mutagenic activity have been demonstrated (14). Although TA showed no mutagenic activity, its presence could result in nitrosamine formation, as has been proposed by Woody and Chu (14). The risk to animal populations arising from continuing low-level exposure to such metabolites must be taken into consideration. The high frequency of natural contamination of sunflower seeds by *Alternaria* metabolites indicates that future studies to prevent contamination are needed.

**ACKNOWLEDGMENTS**

The authors are grateful to the International Foundation for Science and CONICET (Consejo Nacional de Investigaciones Cientificas y Tecnicas Argentina) which supported this research through grants number E/1896-1 and PID-BID Res. N 357-92, respectively. We are also grateful to the oil-seed factory staff who provided us with sunflower-seed samples.

**TABLE 2. Alternaria mycotoxin distribution in whole sunflower seeds, meal, and oil after inoculation**

<table>
<thead>
<tr>
<th>Toxin Strain of <em>A. alternata</em></th>
<th>Whole seeds (100%)</th>
<th>Meal (%)</th>
<th>Oil (%)</th>
<th>Recovery (meal + oil, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOH/539</td>
<td>3.9</td>
<td>3.5 (89.7)</td>
<td>ND$^b$</td>
<td>(89.7)</td>
</tr>
<tr>
<td>AOH/RCY</td>
<td>3.1</td>
<td>2.9 (93.5)</td>
<td>ND$^b$</td>
<td>(93.5)</td>
</tr>
<tr>
<td>AME/539</td>
<td>3.1</td>
<td>1.6 (51.6)</td>
<td>1.4 (45.2)</td>
<td>(96.8)</td>
</tr>
<tr>
<td>AME/RCY</td>
<td>2.7</td>
<td>1.0 (37.0)</td>
<td>1.2 (44.4)</td>
<td>(81.4)</td>
</tr>
<tr>
<td>TA/539</td>
<td>542.6</td>
<td>365 (67.3)</td>
<td>12.4 (2.29)</td>
<td>(69.6)</td>
</tr>
<tr>
<td>TA/RCY</td>
<td>355.8</td>
<td>228 (64.1)</td>
<td>5.8 (1.63)</td>
<td>(65.7)</td>
</tr>
</tbody>
</table>

$^a$ Without inoculation of seeds: no toxins were detected in the samples. $^b$ AOH, alternariol; AME, alternariol monomethyl ether; TA, tenuazonic acid. $^c$ ND, not detected.

**Erratum** to *Journal of Food Protection* Vol. 57 No. 10, October 1994, pages 918-920, "Effect of Honey on Bacterial Growth and Spore Germination". Incorrect figures appeared in this article. The following are the correct figures and their legends as they should have appeared. The editors regret this error and apologize to the authors.

**Figure 1. Effect of honey on germination of spores of *B. cereus*. Columns: White: 0%, Dotted: 1%, Black: 2% honey concentration.**

**Figure 2. Effect of honey on bacterial leakage. Columns: White: S. dysenteriae; Dotted (light): S. dublin; Dotted (heavy): S. aureus; Black: B. cereus.**

**REFERENCES**