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Through a microbiologist's eyes: optimal time to add honey to tea

Worksheet 1

Comparing the antimicrobial activity of different types of honey

What is the agar well-diffusion method?

The agar well-diffusion method is used to investigate whether a substance can inhibit bacterial growth. Wells are made in agar that has been inoculated with bacteria, and the test samples are added to the wells. If a sample inhibits bacterial growth, a clear area called an inhibition zone forms around the well.[1]

A larger inhibition zone generally indicates stronger antimicrobial activity, although the result may also be affected by how well the sample diffuses through the agar.

Measuring the inhibition zone

Measure the total diameter of the clear zone in millimetres across its widest part, through the centre of the well (figure 1). Include the diameter of the well in the measurement. If the zone is irregular, measure it in two perpendicular directions and calculate the average. Record 0 mm if no clear zone is visible.

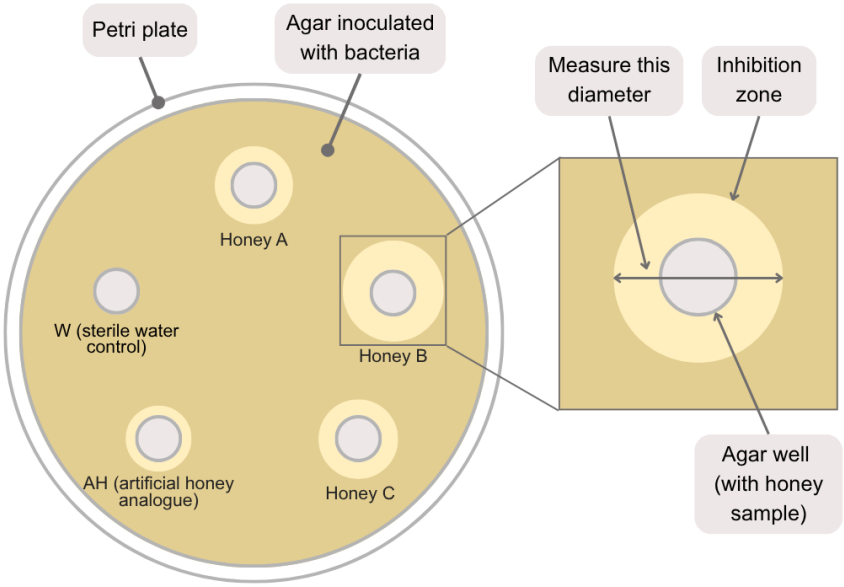


Figure 1: Measuring the diameter of an inhibition zone around an agar well
Image courtesy of the authors

Instructions

1. Perform Activity 1 using the provided protocol (Comparing the antimicrobial activity of different types of honey).
2. Before incubation, record each honey sample, its label, type and source in table 1.
3. After incubation, observe the sealed Petri dish without opening it.
4. Identify the inhibition zone around each honey well.
5. Using figure 1 as a guide, measure the total diameter of each inhibition zone in millimetres across its widest part, through the centre of the well. Include the well in the measurement.

   Note: If a zone is irregular, measure it in two perpendicular directions and calculate the mean. If no clear zone is visible, record 0 mm.
6. Record the measurements and any additional observations in table 2.
7. Compare the antimicrobial activity of the honey samples and select those showing inhibition for use in Activity 2.
8. Summarise the results by answering the questions below:

   a) Which honey produced the largest inhibition zone?

   b) Which honey showed the weakest antimicrobial activity?

   c) Did the sterile water control produce an inhibition zone? What does this indicate?

   d) Did the artificial honey analogue produce an inhibition zone? What does this suggest about the effect of high sugar concentration and osmotic stress on bacterial growth?

   e) How did the inhibition zone produced by the artificial honey analogue compare with those produced by the natural honey samples?

   f) Why was it important to add the same volume of each honey sample?

   g) Why might different types of honey produce different inhibition zones?

   h) Which honey samples should be selected for Activity 2? Explain your choice.

Table 1: Honey samples

Sample	Type of honey	Source or additional information
A		
B		
C		

Table 2: Antimicrobial activity

Sample	Diameter 1 (mm)	Diameter 2, if required (mm)	Mean inhibition-zone diameter (mm)	Additional observations
Honey A				
Honey B				
Honey C				
Water control (W)				
Artificial honey analogue (AH)				

References

[1] Hossain T J (2024) Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. *European Journal of Microbiology and Immunology* **14**: 97–115. doi: 10.1556/1886.2024.00035