Practical activity (9)

Antimicrobial Study of Fruit Juice and Herbal Tea

(Teacher's Guide)

I. Objectives of the experiment

1. To collect and culture microbial samples from the daily environment.
2. To estimate the microbial density of a broth culture by comparing the cloudiness of broth culture with the 0.5 McFarland turbidity standard.
3. To investigate the antimicrobial effect of the selected fruit juice and herbal tea.

II. Expected Learning Outcomes

Upon completion of the activities, students should be able to:

1. culture microorganisms collected from the environment;
2. estimate the microbial density of broth culture by comparing it to turbidity standards; and
3. evaluate the antimicrobial effects of fruit juices and herbal tea according to inhibition of colony formation.

III. Teaching notes:

1. Introduce the task by going through the "Scenario" with the students or have the students read through this part and the "Guiding questions about the design of the experiment" as a pre-class activity.
2. Discuss the design of the experiment with the students using the "Guiding questions about the design of the experiment".
3. Plan the laboratory work with reference to the "Time allocation for the experimental activities" in section IV according to the lesson time of your school.
4. Always remind the students about the safety precautions of each part of the experiment.
5. Go through the "Results" and "Discussion" with the students.

IV. Time allocation for the experimental activities:

Practical work		Duration	
		Within class	**Out of class** (to be done by the laboratory technicians)
Lab session 1: Part (1) of the experiment			
1	Sample collection	30 min	
2	Incubation of microbes		2 days
Lab session 2: Part (2) of the experiment			
1	Estimation of microbial density	15 min	
2	Culturing the microbes in different test solutions	15 min	
3	Incubation of microbes		2 days
Lab session 3: Part (3) of the experiment			
1	Dilution of broth culture and inoculation of microbial samples onto agar plates	30 min	
2	Incubation of microbes (to be handled by laboratory technicians)		2 days
Lab session 4: Part (4) of the experiment			
1	Observation and analysis	30 min	
Total lesson time for the experimental activities		Lab session 1: 30 min Lab session 2: 30 min Lab session 3: 30 min Lab session 4: 30 min	

V. Equipment, materials, and preparatory work for the experiments

A. Part (1) of the experiment: Collection of microbial samples from environmental surfaces

a) Equipment (per group)

No equipment needed

b) Materials (per group)

- Culture tube with sterile LB broth (10 ml) × 1 (per student)
- Sterile swab (breakable stick with a cotton bud) × 1 (per student)
- Culture tube rack × 1
- Masking tape × 1
- Ruler × 1
- Permanent marker × 1
- 70% ethanol in spray bottle × 1
- Paper towel × 1 box
- Biohazard bag × 1
- Disposal container with 10% chlorine bleach × 1

c) Preparatory work

Preparation of LB culture broth **(To be done by the teacher / laboratory technicians)**

1. One day before the lab session, dissolve LB powder in distilled water according to the manufacturer's instructions. Autoclave the solution, and store it properly until use. Before the lab session, aliquot 10 ml sterile LB broth into sterile culture tubes. Prepare one tube per student. Alternatively, ready-to-use LB broth can be purchased from biotechnology companies.

B. Part (2) of the experiment: Estimation of the microbial density of the broth culture and culturing of microbes in different test solutions

a) Equipment (per group)

- Bunsen burner × 1
- Spark lighter × 1
- Micropipettes (P5000 and P1000) and sterile tips

b) Materials (per group)

- LB broth culture from Part (1) × 1 (per student)
- Sterile LB broth (30 ml) × 1
- Apple juice (1.5 ml) × 1
- Pomegranate juice (1.5 ml) × 1
- Honeysuckle flower tea (1.5 ml) × 1
- Ampicillin (25 mg/ml, 1.5 ml) × 1
- Sterile distilled water (1.5 ml) × 1
- 0.5 McFarland turbidity standard × 1
- Cardboard with black and white strips × 1
- Culture tube rack × 1
- Micro-centrifuge tube rack × 1

- Permanent marker × 1
- 70% ethanol in spray bottle × 1
- Paper towel × 1 box
- Biohazard bag × 1
- Disposal container with 10% chlorine bleach × 1

c) Preparatory work

Preparation of LB broth and aliquot of test solutions **(To be prepared by the teacher / laboratory technicians)**

For LB broth:

1. One day before the lab session, dissolve LB powder in distilled water according to the manufacturer's instructions. Autoclave the solution, and store it properly until use. Before the lab session, aliquot 30 ml LB broth into sterile bottles. Prepare one tube per student. Alternatively, ready-to-use LB broth can be purchased from biotechnology companies.

Preparation of the 0.5 McFarland turbidity standard:

1. Prepare a 1% w/w barium chloride ($BaCl_2$) solution by dissolving 0.01 g $BaCl_2$ in 0.99 g distilled water.
2. Prepare a 1% v/v sulfuric acid (H_2SO_4) by adding 1 ml H_2SO_4 to 99 ml distilled water.
3. Thoroughly mix 0.5 ml of 1% barium chloride solution with 99.5 ml of 1% sulfuric acid.
4. Aliquot 10 ml standard solution into culture tube. Prepare one tube per group.
5. When the solution is not in use, store it at room temperature and wrap the tube with aluminium foil. Prepare a new standard after a storage for 6 months.

For apple juice and pomegranate juice:

(Please note that the fruit juice should be fleshly prepared or bottled juice that has no preservatives added should be purchased for use.)

1. Before the lab session, aliquot of 1.5 ml fruit juice into sterile micro-centrifuge tubes. Prepare one tube for each fruit juice per group.

For honeysuckle flower tea:

1. Soak 50 g raw honeysuckle flower in 200 ml distilled water for 30 minutes.
2. Boil the mixture for 45 minutes. Remove the residue and store the mixture properly at 4°C until use.
3. Before the lab session, aliquot 1.5 ml extract into sterile micro-centrifuge tubes. Prepare one tube per group.

For ampicillin solution:

1. Dissolve 250 mg ampicillin powder in 10 ml sterile distilled water. Mix the solution by turning the tube upside down a few times. Store it properly at 4°C until use.
2. Before the lab session, aliquot 1.5 ml ampicillin solution into sterile micro-centrifuge tubes. Prepare one tube per group.

d) Sterilisation and disposal

After the experiment, some equipment and materials to discard (solid or liquid) should be sterilised by steam under pressure (autoclave) at 121°C, 15 psi pressure for at least 30 minutes or immersed in 10% chlorine bleach for at least 2 hours.

C. Part (3) of the experiment: Antimicrobial study of fruit juice and herbal tea

a) Equipment

- Bunsen burner × 1
- Spark lighter × 1
- Micropipettes (P5000, P1000, and P200) and sterile tips

b) Materials

- Microbial broth culture from Part (2) × 5
- Culture tube rack × 1
- LB agar plates × 5
- 15 ml centrifuge tube × 10
- Centrifuge tube rack × 1
- Sterile LB broth (200 ml) × 1
- Inoculating loop × 1

- Permanent marker × 1
- 70% ethanol in spray bottle × 1
- Paper towel × 1 box
- Biohazard bag × 1
- Disposal container with 10% chlorine bleach

c) Preparatory work

Preparation of LB agar plates, aliquot of distilled water and preparation of *E. coli* culture broth **(To be prepared by the teacher / laboratory technicians)**

1. One day before the lab session, dissolve LB agar powder in distilled water according to the manufacturer's instructions. Autoclave the solution. Pour into Petri dishes (15–20 ml per dish). Wrap the dishes with parafilm, and store them properly at 4°C until use. Prepare 5 LB agar plates per group. Alternatively, ready-to-use LB agar plates can be purchased from biotechnology companies.

2. Before the lab session, aliquot 200 ml sterile LB broth into sterile bottles. Prepare 1 bottle per group.

3. One day before the lab session, check the turbidity of the students' sampling culture broth by comparing it with the 0.5 McFarland standard. If the turbidity of all the broth culture of the same group is much lower than that of the standard solution, which means not having a sufficient number of bacteria, then an *E. coli* broth culture with designated turbidity should be provided to that group (for details, please refer to Step 4). If the turbidity of all the broth culture of the same group is much higher than that of the standard, dilute one of the culture by adding sterile culture broth until similar turbidity is obtained.

4. An overnight culture of *E. coli* should be provided to the group which do not obtain a sufficient number of bacteria in their sampling broth culture for the completion of the subsequent experimental procedures. Grow a tube of *E. coli* culture (Sigma-Aldrich or Promega) in an appropriate broth medium (e.g. LB broth) for about 16 hours. Make a 1:5 dilution of the overnight culture of *E. coli* cells (1 ml *E. coli* culture with 4 ml sterile LB broth). Prepare 1 tube of diluted *E. coli* culture per group as needed.

   Sigma-Aldrich distributor in Hong Kong: Tin Hang Technology; Tel: 2817 2121
   Website: www.tinhangtech.com/home/

   Promega distributor in Hong Kong: Bio-Gene Technology Ltd; Tel: 2646 6101
   Website: www.bio-gene.com.hk

d) Sterilisation and disposal

After the experiment, some equipment and materials to discard (solid or liquid) should be sterilised by steam under pressure (autoclave) at 121°C, 15 psi pressure for at least 30 minutes or immersed in 10% chlorine bleach for at least 2 hours.

D. Part (4) of the experiment: Counting the colonies and analysing the results

a) Equipment

- Mobile device × 1

b) Materials

- LB agar plates from part (3) × 5
- Permanent marker × 1
- 70% ethanol in spray bottle × 1
- Paper towel × 1 box
- Biohazard bag × 1
- Disposal container with 10% chlorine bleach × 1

c) Preparatory work

No specific preparation work required

d) Sterilisation and disposal

After the experiment, some equipment and materials to discard (solid or liquid) should be sterilised by steam under pressure (autoclave) at 121°C, 15 psi pressure for at least 30 minutes or immersed in 10% chlorine bleach for at least 2 hours.

VI. Suggested answers to the "guiding questions about the design of the experiment" and Table 2

1. What equipment is suitable for the collection of microbiological samples from the environment?

   A swab stick with a cotton bud and culture broth

2. How can we estimate the microbial density of a broth culture without a sophisticated instrument?

   We can compare the turbidity / cloudiness of the broth culture with some standards, e.g. 0.5 McFarland turbidity standard. The turbidity of 0.5 McFarland standard is comparable to a bacterial suspension with 1.5 × 10^8 CFU/ml.

3. If a test drink is claimed to be antimicrobial, what do we expect to observe on the agar plates after the microbial incubation period?

   We expect to observe very few microbial colonies on the agar plates, as the antimicrobial agents can inhibit the growth of microbes.

Table 2

	Tube A	Tube A1	Tube A2	Tube A3
Dilution fold	0	100-fold dilution of tube A	100-fold dilution of tube A1	10-fold dilution of tube A2
Dilution fold of the microbial broth culture	*0*	*100*	*10,000*	*100,000*

VII. Results

1. The choice of your sampling environmental surface is

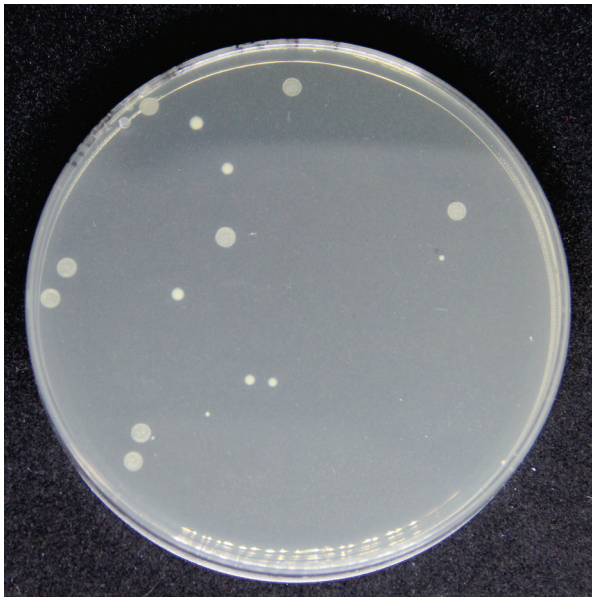
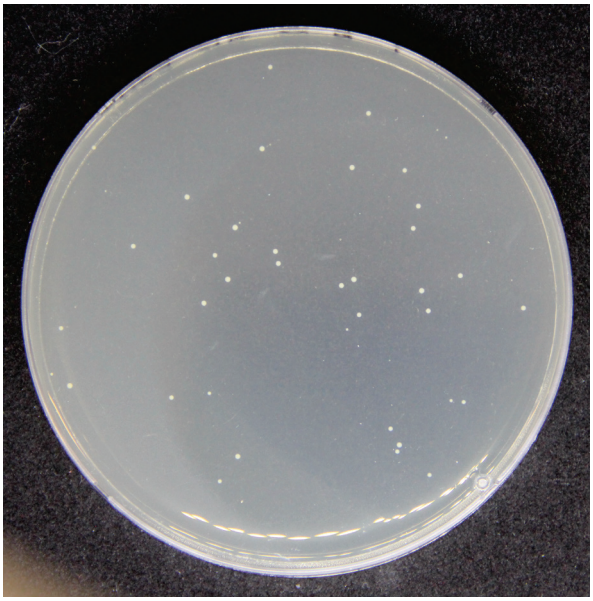
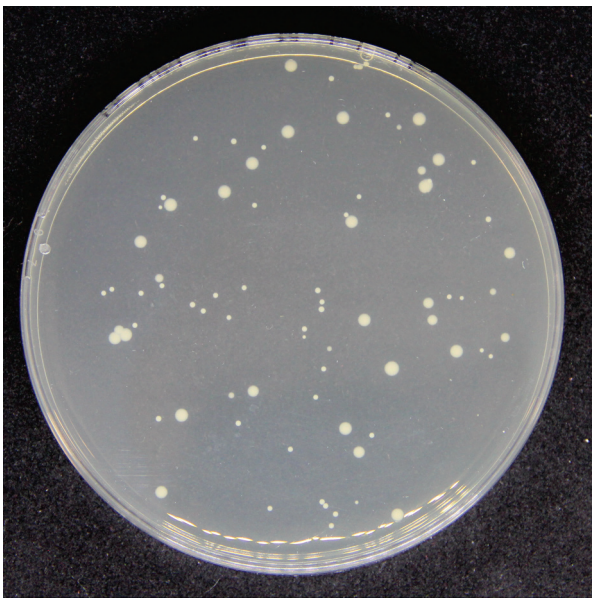
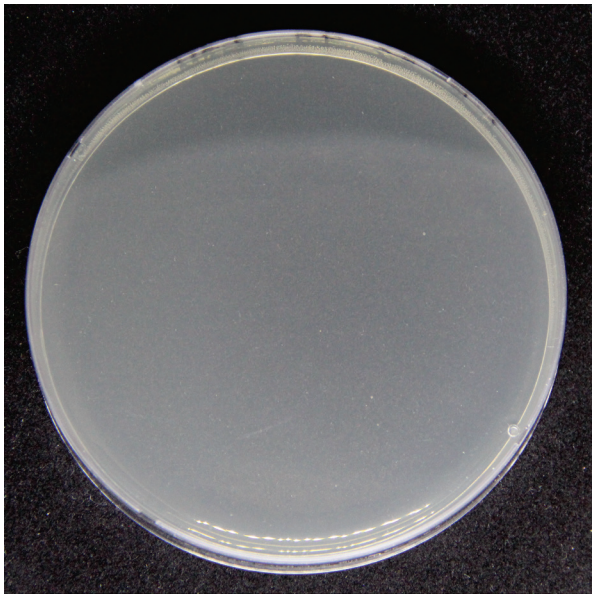
   _____, and the sampling area is _____ cm^2.

2. Record of the microbial samples collected and the turbidity of the broth culture of the group.

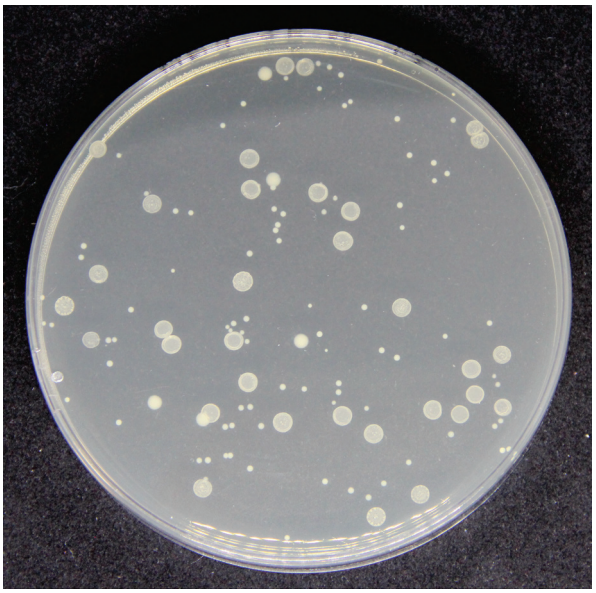
Name of member	Sampling environmental surface	Area of the sampling surface (cm^2)	Turbidity of the broth culture as compared with the 0.5 McFarland turbidity (higher / similar to / lower)	Whose broth culture is selected for further culturing in different test solutions? (Put a "✓".)

3. Paste the photo or draw a picture of the LB agar plates after incubation in the respective boxes below.

[For reference only. The results may vary due to different concentrations of fruit juice.]

"A" Apple juice 	"P" Pomegranate juice 
"H" Honeysuckle flower tea 	"Amp" Ampicillin solution 

“W” Sterile distilled water



4. Record the number of CFU on the LB agar plates in the following table.

Test solution	Number of CFU
Apple juice	*15*
Pomegranate juice	*41*
Honeysuckle flower tea	*83*
Ampicillin solution	*0*
Sterile distilled water	*129*

Discussion

1. Suggest a reason why we have to make dilutions of the microbial broth culture before plating.

   We want to obtain the colony-forming units (CFU) found on the plates to be within the countable range (25–250) after incubation.

2. The number of colony-forming units (CFU) shows a positive correlation with the growth of the microbes. Thus, which test solution allows the most growth of microbes? Which test solution provides the best antimicrobial effect?

   The distilled water allows the most growth of microbes. The ampicillin solution provides the best antimicrobial effect.

3. Based on the rationale of question 2 and the experimental data, arrange the antimicrobial property of the test fruit juices and herbal tea in descending order.

   [For reference only]
   Apple juice > pomegranate juice > honeysuckle flower tea

4. Ampicillin solution and sterile distilled water served as controls in this experiment with different functions. State the rationale for setting these two different controls.

   Ampicillin is a positive control which has a known antimicrobial effect. Distilled water is the negative control that does not have any antimicrobial effect.

VIII. References

Holland, K. T. (1989). Microbiological sampling techniques. In M. W. Greaves & S. Shuster (Eds). *Pharmacology of the skin II. Handbook of experimental pharmacology*, *87*(2). Berlin and Heidelberg: Springer.