

Microbiology for the Health Sciences Laboratory

Lab Session 10 - Quantitation and Characterization of Microorganisms in Food

Introduction

Microorganisms have several types of relationships with food. Microorganisms are used to produce food (largely due to the prolific fermentative metabolism of specific bacteria), they can initiate the processes involved in food spoilage (largely due to their prolific reproduction and the role of specific bacteria as decomposers), and they can be a source of human disease (if food is contaminated with microorganisms that can infect humans or if they produce toxins that can be ingested). Unfortunately, infectious or toxic doses of microorganisms can be present in foods before the food is recognizable as spoiled. Foods can contain 10^5 to 10^6 mo's per gram without being recognized (i.e. physical or sensory characteristics) as spoiled. The purpose of this lab is to determine the number and diversity of microorganisms in various food substances.

A. Review the following for reference:

1. Text: Chapter 31: Pgs 748-750 and 756-759.

B. Do the following during lab:

Preparation of Food Sample:

1. Collect a 1 ml or 1gm sample of the food:
 - a. Solids: Use a spatula and weigh out a one gram sample of food.
 - b. Liquids: Use a 1 ml pipet to collect a 1 gram sample.Note: Use liquid substances where possible. Assume 1ml = 1 gm.
2. Transfer the food sample to a 9 ml water blank. This is a 1:10 dilution.
Note: It may be necessary to homogenize or finely slice meats or other solids.
3. Cap the tube tightly and shake it until your arm becomes weak. Allow the solids to settle and the fats to 'float'.
You now have, from a 1 gram sample of food, a suspension of microorganisms that were present in the original food sample, assuming that the mo's were detached from the food by shaking.
Note: Use the intermediate layer, the water phase (not the solids or the fats) to inoculate plates as follows:

Inoculation of Culture Media:

1. Use one plate of each of the following culture media for each food.
Inoculate the media with 0.1 ml (this is another 1:10 dilution) of the prepared food sample:

Culture Media	Purpose
Trypticase Soy Agar	Total number of microorganisms in food.
Columbia Agar	Total number of gram positive organisms.
Mannitol Salt Agar	Isolation and identification of staphylococci including <i>S. aureus</i> .
Eosin-Methylene Blue Agar	Isolation and identification of coliforms, especially <i>E. coli</i> .
Hektoen Agar	Isolation and tentative identification of various gram negative mo's including <i>Salmonella sp.</i> , <i>Shigella sp</i> and <i>Proteus sp</i> .

2. Spread the 0.1 ml inoculum over the surface of the plate with a swab to make a lawn of growth.
Note: You can use one swab to do all 5 plates.
3. Incubate at 37 C for 24-48 hours.
4. Evaluate any microbial growth that occurs.

C. Techniques:

The following is a method for determining the precise microbial content of foods:

Quantitative analysis of microorganisms in raw food

1. Weigh 1 gram of food (e.g. hamburger) and blend with 9 ml of 0.9% sterile water for 3 minutes.
2. Do 2 to 4, 10-fold dilutions of the supernate (1 ml + 9 ml saline).
3. Plate 0.1 ml of the dilutions on the appropriate culture media.
4. Count colonies to determine the exact number of mo's per gram of food.

Qualitative analysis of microorganisms in raw food

1. Isolate colonies of suspected pathogens.
2. Use appropriate biochemical tests to identify agents.

D. Results:

1. Estimate the number and type of microorganisms in the prepared food sample:
 - a. Number of colonies: Colonies counted x 100 (the dilution factor) = # of mo's per gram of food.
 - b. Tentative identification based on growth and appearance on selective/differential media:
Staph.sp., *E. coli*, *Salmonella sp.*, *Shigella sp* and *Proteus sp*.

2. Comment on the microbial content of the food.

FOOD CHOSEN: _____

SOURCE: _____

Physical Characteristics of Food:

(Note: Describe the physical and sensory characteristics of the food)

Total Microbial Content of Food:

Use the TSA plate. Count colonies if possible. Estimate number of colonies if there are too many to count.

Microbial Content and Tentative Identification of Microorganisms in Food:

Columbia Agar

Mannitol Salt Agar

Eosin-Methylene Blue Agar

Hektoen Agar

E. Discussion and Conclusions: