Isolation and Characterization of Bacteria from Yogurt

Introduction

The term "probiotics" has received a great deal of attention in recent years. History, however, attributes the discovery of a probiotic bacterium, specifically *Lactobacillus bulgaricus,* and its potential for enhancing health, to studies conducted in the early 1900s. In 2002, recognizing the increased interest in amending food products with probiotic microbes, the United Nations and the World Health Organization form a working group to draft guidelines for evaluating such products. This group formally defined a probiotic as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host". In short, probiotics have been commercialized to generate numerous products aimed towards increasing one's health. Such products include yogurt, fermented foods, dietary supplements, and beauty aides.

One of the more readily recognizable probiotic products is yogurt manufactured and sold by the Danone North America Corporation under the brand name Activia®. As of 2024, the company claims to produce "the only yogurt products in the United States with their formulation that includes a probiotic culture called *Bifidobacterium animalis lactis* DN-173 010/CNCM I-2494" (https://www.activia.us.com/frequently-asked-questions/). [Note: As is typical of commercial firms, microbial names are often incorrectly presented. In the present case, the above bacterium is more properly known as *Bifidobacterium animalis* subspecies *lactis* strain DN-173 010/CNCM I-2494.] This probiotic bacterium is known to have positive health affects (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4858658/). In addition to *Bifidobacterium*, Activia yogurt also contains three other live cultures that certainly contribute to the making of the product if not also serving as a probiotic. Selected attributes of these four bacterial species, which are all Gram positive, are presented in Table 1.

Materials Required

- Activia® yogurt
- BEA agar plates
- EZ Anaerobe Pouch System (Cat. No. BD 260683; Becton-Dickinson)
- EZ CO_2 Pouch System (Cat. No. BD 260684; Becton-Dickinson)
- Mineral Oil
- Moeller Arginine Decarboxylase broth tubes
- MRS agar plates (Cat. No BD 288210; Difco, Becton-Dickinson)

Procedure

Isolation of Yogurt Bacteria

1) Label one plate of MRS agar as "+O_2", another as "-O_2", and a third as "+CO_2".
2) Use a sterile microbiological loop to place a loopful of Activia® yogurt onto each of the labeled MRS agar plates. With a sterile loop, use the streak plate method to spread the yogurt sample across the agar surface.

Table 1.

Bacterial Species	Lifestyle	Morphology	Esculin Hydrolysis	Arginine Hydrolysis
Bifidobacterium animalis subspecies *lactis* strain DN-173 010/CNCM I-2494	Anaerobe	Irregular rods with the central portion sometimes enlarged; cells occur in pairs or as branches to form cross-like aggregates of four cells	Positive	Negative
Lactobacillus bulgaricus (more accurately *Lactobacillus delbruekii* subspecies *bulgaricus*)	Anaerobe	Rod with rounded ends, single or short chains	Positive	Negative
Lactococcus lactis	Facultative Anaerobe	Cocci in pairs or short chains	Positive	Positive
Streptococcus thermophilus	Facultative Anaerobe	Cocci in short to long chains	Negative	Negative

3) Use a loop to smear a small amount of Activia® yogurt onto a glass slide. The smear should approximate the size of a quarter. Allow the slide to air dry. This smear will be stained as described below.
4) Label one resealable GasPak™ EZ Pouch as "+O_2", another as "-O_2", and a third as "+CO_2".
5) To each labeled pouch, place the appropriate inoculated MRS agar plate inside.
6) Remove an Anaerobic BD GasPak™ EZ Pouch System Sachet from the outer foil packaging. Place it in the resealable pouch with the inoculated MRS agar plate labeled "-O_2". The sachet should be placed between the plates and the pouch.

   Note: On the one side of the sachet is a white pill. This is the anaerobic indicator. This pill should remain white throughout the incubation period. If the pouch loses its anaerobic environment, the pill will turn blue in color.
7) Remove a Carbon Dioxide BD GasPak™ EZ Pouch System Sachet from the outer foil packaging. Place it in the resealable pouch with the inoculated MRS agar plate labeled "+CO_2". The sachet should be placed between the plates and the pouch.
8) Seal the "-O_2" and "+CO_2" pouches by pressing the zipper part of the pouch together. Do not seal the pouch labeled "+O_2".
9) Incubate all three pouches at 37°C for 36-48 hours.

10) Remove the pouches from the incubator and record the appearance of bacterial colonies on each of the plates incubated under the different environmental conditions.
11) Appropriately discard of the BD GasPak™ EZ Pouch System Sachet and pouch.

Staining of Isolated Yogurt Bacteria

1) For each colony type noted on the different MRS agar plates, label a glass slide and place a small (about 10 µL) drop of distilled water in the center.
2) For each colony type, use a sterile toothpick to remove colony from the surface of the agar plate. Place the toothpick in the water drop and proceed to make a smear about the size of a quarter. Allow the slide to air dry. Note: Smears of very small colonies may require that more than one be removed from the agar surface.
3) Heat fix the smear.
4) Cover the fixed smear with Gram's Crystal Violet for one (1) minute.
5) Gently rinse off the stain with distilled water.
6) Blot the glass slide dry with paper towel.
7) Using the oil immersion objective of the microscope, observe and record the cell types on each slide.

Biochemical Characterization of Isolated Yogurt Bacteria

A. Esculin Hydrolysis

For each colony type noted on the different MRS agar plates, streak one half of a BEA plate. Incubate the plate at 37°C for 24-48 hours. The production of a dark pigment in the medium surrounding the bacterial growth is indicative of esculin hydrolysis.

B. Arginine Decarboxylation Assay

1. For each organism to be tested, obtain one (1) arginine decarboxylation broth tube [ADC].
2. Using a loop, inoculate the ADC broth tubes with the organism to be tested.
3. Using a sterile, plastic bulb-type transfer pipette, layer the surface of all the broths with a small volume (about 0.5 ml) of sterile mineral oil. DO NOT MIX!
4. Incubate all the tubes for at 37°C and observe up to four (4) days or more.
5. Possible results:
    a. If the inoculated medium is yellow, the organism is decarboxylase-negative for arginine.
    b. If the inoculated medium turns/remains purple, the organism is decarboxylase-positive for arginine.

    IMPORTANT NOTE: A decarboxylase-positive organism may turn the broth yellow at first before returning to a purple color. Therefore, it is important to monitor the color for at least four (4) days.

Yogurt Bacteria Lab Report

Name _____

For each of the unique isolates collected from yogurt during this exercise, ***list*** the characteristics (morphological, biochemical, etc.) that each possesses. Based upon these qualities, suggest an identification from the information provided in Table 1.

