

Microbiology Experiment #7 – Exoenzymes, Differential and Selective Media

Every microorganism has different metabolic capabilities. By comparing these capabilities, one can group and identify the organisms being studied. Microorganisms are metabolically quite diverse. Consequently, they are capable of degrading a variety of organic molecules. These molecules can be categorized by their structure and size. Some commonly utilized organic molecules are either macromolecules or constituents of macromolecules. The classes of organic macromolecules include proteins, carbohydrates, lipids or fats, and nucleic acids. Each of these macromolecules is composed of individual, smaller monomeric units. The proteins are composed of amino acids held together by peptide bonds. Monosaccharides in ether linkages comprise the building blocks of carbohydrates. The simple lipids or fats have glycerol and fatty acids in ester linkages as their basic structure. Nucleic acids are composed of individual nucleotides (sugars, bases, and phosphates).

In addition to having to confront the various classes of molecules, bacteria must also deal with molecules that vary in size or molecular weight. The first task that confronts a microorganism when it is exposed to a potential nutrient is the task of transporting that molecule into the cytoplasm. The larger-molecular-weight molecules are incapable of permeating the cytoplasmic membrane. Therefore, to utilize large macromolecules such as proteins and carbohydrates as sources of energy, microorganisms must first release specific enzymes called **exoenzymes** to start the extracellular breakdown of these molecules by **hydrolysis**. Following the extracellular hydrolysis of macromolecules, transport proteins embedded in the cytoplasmic membrane import the simple monomers for the cell to metabolize. In the procedures that follow, the activities of various exoenzymes that may be produced by isolated experimental microorganisms are investigated.

Starch is a polymer made up of amylose, a polymer of glucose units and amylopectin. Amylopectin is a branched polymer containing phosphate groups (Figure 21.1). Bacteria capable of hydrolyzing starch produce the enzyme **amylase**. This enzymatic hydrolysis forms shorter polysaccharides called **dextrins**, which ultimately can be hydrolyzed to yield disaccharides and monosaccharides. These end products are transported into the cell and used in biosynthetic reactions (e.g., formation of cell wall components) or energy-yielding reactions (e.g., glycolysis or TCA cycle reactions).

Casein is the high-molecular-weight protein found in milk. As with starch, this macromolecule must be hydrolyzed by exoenzymes into smaller molecules. In this case, amino acids are formed and are capable of being transported through the cell membrane. This hydrolysis is carried out by a proteolytic exoenzyme known as **caseinase**.

Upon boiling collagen, a major connective tissue, the product **gelatin** is formed. Certain bacteria are capable of producing a proteolytic exoenzyme called **gelatinase**, which hydrolyzes the protein to amino acids. These amino acids can then be transported into the cell for further metabolism. At temperatures below 25°C, gelatin will remain a gel, but if the temperature rises about 25°C, the gelatin will be liquid (remember what happens to Jell-O at room temperature). If you cool the liquefied gelatin, it will resolidify. Gelatin hydrolysis has been correlated with pathogenicity of some microorganisms. It is thought that pathogenic bacteria may break down tissue and spread to adjacent tissues.

The ability of certain microorganisms to degrade lipids such as triglycerides occurs through the action of exoenzymes called **lipases**. These enzymes cleave the ester bonds in the molecules, leading to the formation of glycerol and fatty acids. These degradation products can be taken up by microorganisms and metabolized aerobically for energy, or can be used in the biosynthesis of other fats or cellular components. Some organisms produce a lipase that can hydrolyze Tween 80 (the detergent polyoxyethylene sorbitan monooleate) into oleic acid and polyoxyethylated sorbitol.

DNase activity refers to the ability of bacteria to hydrolyze DNA in the growth medium to its component nucleotides. Presumably, these organisms would also secrete a phosphatase to convert the nucleotides to nucleosides for transport into the cells. After two days of bacterial growth on DNA agar, the plate is flooded with 1 M HCl, which causes the DNA to come out of solution and turn the medium opaque. A clear zone around the colonies indicates that the DNA has been hydrolyzed.

DNA agar = TSA + 2g/L DNA (Krieg, 1994)

Interpretation: after adding 1 M HCl --> clear zone in medium = + ; no clear zone = -

Procedure

1. Draw lines on the bottoms of the available starch, milk, DNA, and Tween agar plates such that each plate is divided into 2 sectors. Label each sector with name of your known or unknown organism.
2. Streak your plates for single colonies and incubate at room temp until the next lab meeting.
3. Inoculate 2 liquified gelatin tubes (37C) with your known or unknown microbe using an inoculating loop, incubate at RT until the next lab meeting.
4. Collect results.
 - Amylase – flood the starch plate with iodine solution, which reacts with starch in the medium to produce a brown-black color. If the organism has hydrolyzed the starch (a positive result), there will be a localized clearing around colonies.
 - Caseinase – observe the area around colonies on the milk plate for a loss of white color. In the absence of casein, the medium becomes more translucent.
 - DNase – flood plate with 1 M HCl to precipitate DNA in the medium. Observe the area around colonies on the DNA plate for a loss of opacity due to hydrolysis of DNA in the medium.
 - Gelatinase – Place gelatin tubes into an ice bucket for 5 minutes. Remove tubes from ice and tilt at a 30° angle to determine whether gelatin has been liquified (a positive result.)
 - Tween – Look for precipitate around growth = +
 - Blood Agar- look for clear zone (β-hydrolysis) or greenish zone (α-hemolysis) around bacteria

	Amylase (starch)	Caseinase (milk)	DNase	Gelatinase	Tween Hydrolysis	Lecithinase	Hemolysis
Known							
Unknown							

Selective and Differential Media

<p>EG medium is a defined minimal medium consisting of 1x E salts + 0.5% glucose. E salts contain only MgSO₄, K₂HPO₄, Na₂NH₄PO₄, and citric acid, and therefore, in order to grow, an organism must be prototrophic (capable of synthesizing, opposite is auxotrophic) for all necessary molecules such as amino acids, nucleotides and vitamins.</p> <p><u>EG minimal medium (1 L)</u> 955mL dH₂O 20mL 50x E salts for plates, add 16g purified agar (standard agar has too many contaminating nutrients for minimal medium) after autoclaving, add 25 mL 20% glucose (autoclaved separately)</p>	<p><u>50x E salts (1 L)</u> 670 mL dH₂O 10g MgSO₄ * 7H₂O 100g citric acid 500g K₂HPO₄ 175g Na₂NH₄PO₄</p>
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Interpretation: Growth = + ; no growth = -

2. **Brilliant Green Bile** - Enzymatic Digest of Gelatin is the carbon and nitrogen source used for general growth requirements in Brilliant Green Bile Broth 2%. Oxbile and Brilliant Green inhibit Gram-positive bacteria and many Gram-negative bacteria, other than coliforms. Lactose is a carbohydrate source. Bacteria that ferment lactose and produce gas are detected.

Interpretation: Growth = + ; no growth = - ; Growth with black color in medium = + black

3. **Eosin Methylene Blue medium (EMB)** is frequently used to detect coliform bacteria during water quality testing. Fermentation of lactose or sucrose decrease the pH of the medium which allows uptake of the eosin-methylene blue dye, creating a dark blue-purple color. E. coli will often form colonies with a green metallic sheen due to the rapid acidification of uptake of the dyes.

Interpretation: Growth = + ; no growth = - ; Growth with dark colonies = + dark ; Growth with green colonies = + green

Pseudomonas Agar P & F – These differential media are formulated to enhance the production of the fluorescent pigments pyocyanin (blue) and fluorescein (green) in the genus Pseudomonas.

	EG	BGB	cetrimide	EMB	DOC	PP	PF	
Known								
Unknown								