

Microbial Bioassay

A number of approaches have been historically used to find leads for new and potentially useful biologically active natural products. Today, the drug-discovery process involves a sophisticated array of biological assays, or “bioassays,” which range from live animal tests to cell culture methods to enzyme assays. These assays are typically first used to identify a bioactive crude extract, then applied again as the mixture is purified, such as by chromatographic means, to correlate the activity with one particular substance. This “bioassay-guided fractionation” yields a pure sample of a molecule that an organic chemist analyzes for structure and that pharmacologists investigate for use in a medicine.

One of the simplest assays for antimicrobial activity is the spot disk assay. An aliquot of a test solution is applied to a filter paper disk, then placed on an agar plate that has been preinoculated with a test microbe. A clear area of no growth around the disk, or the zone of inhibition, indicates the presence of an antimicrobial substance in the disk saturated by the test extract.

Objectives

(Record this in your research notebook.)

- Describe the steps involved in the isolation of a natural product.
- Describe and practice sterile microbial techniques.
- Be able to carry out a microbial bioassay.
- Describe a positive and negative microbial bioassay result.
- Understand the use of positive and negative controls.
- Carry out a research project on unknown plant specimens.

Procedure

Day 1 – Extraction and cell culture

- Make a voucher sample of your specimen (note location, date, taxonomic identification).
- Make a 25 percent weight by volume plant/solvent mixture.
- Cover with aluminum foil, label, and place overnight in a dark environment.
- Inoculate a cell culture (bacteria or yeast) in a sterile culture tube, and place in a 37°C incubator.

Procedure

Day 2 – Extraction drying and cell inoculate

- Decant extract (solvent) into clean beaker.
- Introduce sodium sulfate (desiccant), allow to sit for 10 minutes, decant sample into labeled Epitube, cover with aluminum foil, and store.
- Use sterile forceps, dip an assay disk into extract, remove, and place in aluminum boat (label boat). Put boat into an incubator to dry.
- Using sterile technique, introduce 50ml of a 24-hour-old cell culture onto agar plate, spread to create an even distribution (lawn).
- Allow culture to soak into agar surface for at least 10 minutes.
- Using sterile forceps, place positive, negative, and extract disks onto the agar plate.

Procedure

Day 3 – Read bioassay plate

- Read results and record a drawing of what the agar plate looks like in your research notebook.
- If your compound shows a zone of inhibition (halo), measure the distance from the rim of the extract disk to the outer margin of the halo. Use the table below.
- Answer these questions:
 - What is the study of a natural product?
 - What is a bioassay?
 - Why is it necessary to mass out the sample and measure out the volume of alcohol?
 - What is an extraction?
 - What is a voucher? Why is it important to document samples in this manner (i.e., voucher specimen)?
 - Why is it necessary to use a positive and a negative control when performing a microbial assay? What are the expected results of the respective controls (i.e., in terms of zones of inhibition, a.k.a. halos)?
 - What does it mean if a halo appears around an experimental assay disk?
 - If a halo does not appear around the disk, does this necessarily mean that the natural product is not active? Explain.
 - If a halo is present, what is the next step in the drug-discovery process? Explain.

Spot disk array (area of halo)	Qualitative results	Quantitative results
Negative control		
Positive control		
Unknown		