TECHNOLOGY NETWORKS

BACTERIAL CULTURE

There are lots of reasons to culture bacteria, many sources from which to culture them, and diverse species with equally diverse growth requirements – some pickier than others. The picture becomes even more complicated when trying to isolate one or more strains from mixed populations where some species will outcompete others.

To give yourself a fighting chance at successful bacterial culture it is therefore vital to pick the correct culture conditions for your experiment and avoid contamination by using good aseptic technique.

This infographic takes a look at some key points to consider when culturing bacteria.

WHY DO WE CULTURE BACTERIA?

To isolate a particular strain from a mixed sample
e.g. to diagnose the cause of infection
Swabbing a wound and isolating the causative agent from the normal skin flora

To determine antibiotic sensitivity
e.g. to inform treatment
Having isolated the problematic bacterial strain, determining which antibiotics are appropriate for treatment is key

When genetically manipulating bacterial strains
e.g. when making gene mutations or deletions
A vital step in molecular biology for basic research to understand gene function and vaccine design amongst other areas

Using bacteria as tools in molecular biology
e.g. overproduction of a desired protein
Insertion of a protein coding gene under a strong promoter into a lab strain of *Escherichia coli* can turn it into a mini protein factory

For producing foods and beverages
e.g. fermentation, cheese production and probiotics
The action of bacteria on foodstuffs, like cheese, are what give them their character, and can be beneficial to our body's health

HOW DO WE CULTURE BACTERIA?

Growth conditions

Not all bacterial species grow optimally at the same temperature, so it is important to know the right conditions for the species you are trying to culture. Oxygen requirements vary too with some preferring aerobic and others anaerobic environments. Some may also grow better in a humidified environment so choose the correct incubator type.

Nutrient requirements differ between bacterial species, governed by the nutrient acquisition and utilization gene loci they have. It is therefore important to choose a media (whether culturing in liquid or solid media) which supplies everything your target species requires.

Nutrient agar

Often used if culturing a primary diagnostic sample, e.g. a swab, so the bacteria present can be visualized and target species isolated. Also used when trying to enumerate or isolate individual colonies.

If culturing from a liquid, a small amount is normally spread evenly across the plate. If from a swab or other solid, a small patch of the agar plate, called a *butt*, is normally spread from the original material, from which material is drawn with a loop to produce ever decreasing colony densities, enabling single colony isolation.

Liquid broth culture

A small amount of starting material (normally a single colony) is picked into fresh growth media and incubated in optimal growth conditions.

Typically used once a pure culture has been obtained to bulk up bacterial numbers for downstream applications, such as DNA extraction, food production, or during genetic manipulation.

Antibiotic sensitivity

In order to treat infections effectively, especially with rising levels of antibiotic resistance, it is important to select an antibiotic that will kill the causative bacterial strain effectively. A bacterial *lawn* is cultured on agar on which antibiotic-infused discs are placed – a clear margin indicates sensitivity to the respective antibiotic.

Selective media

Selective media can be useful for identifying the species being grown as many produce a quickly identifiable color change according to the chemicals that can be broken down or converted by the bacterium.

TIPS FOR GOOD ASEPTIC TECHNIQUE

Speed is of the essence – the swifter you work the less time for contamination.

No double dipping! Avoid transfer of one sample to other samples and reagents by using clean tips, loops and spreaders.

Try not to breathe all over your open culture – you are a source of contamination!

Keep containers open for as **short a time as possible.**

Use sterile disposables or if not **ensure all equipment is sterilized** thoroughly and free of detergents.

Prevent contaminants from the atmosphere (and you) entering the sample by opening it **in a hood or next to a flame.**

Always wear clean gloves – hands are a veritable metropolis of bacteria.

Aliquot stock solutions or media to avoid contaminating a whole batch of reagent.

Make sure your work area is clean and tidy – spraying with a 70 % IMS solution should remove most bacteria.