GloriousMicrobialMedia Portrait Gallery: Bromocresol Purple Lactose Broth

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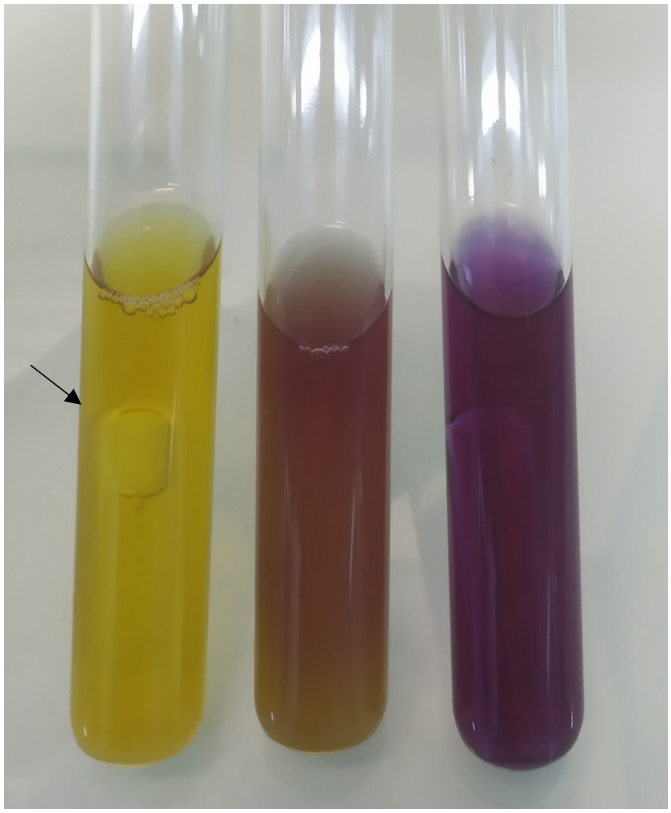

Bromocresol Purple Lactose Broth cultures. Left: fermentation with acid plus gas production (accumulated in the Durham vial: arrow). Middle: fermentation with weak acid but no gas production. Right: negative/uninoculated.

Why do we use it and what does it reveal?

All parts of the biosphere are connected. One of the consequences of this is that dangerous contaminants can more or less readily move around in the environment. To prevent this, we try to build *barriers* close to the source of the contaminants that block their dispersion. We also need good detection systems to check on how effective our barriers are.

One type of hazardous material is faeces, because intestinal pathogens that cause diarrhoea and other tummy troubles are present in high numbers in faeces. If there is no barrier, faeces contaminate environmental water bodies like rivers, lakes and groundwater and, if the water is used for drinking, the faecal pathogens can infect us. This is called the oral-faecal route of infection. In many, but not all, parts of the world there are different hygiene systems – the *barriers* – in place to prevent faecal material entering the environment and contaminating water supplies, especially wastewater treatment plants that treat our sewage, and drinking water purification systems. While these systems are extraordinarily effective barriers that prevent the spread of faecal pathogens in the environment, no system is 100% effective all of the time. For example, waterwater treatment plants can be overwhelmed by storm events, during which they may release untreated sewage into the environment. So we need to monitor water quality to make sure that everything is okay.

The way we do this is to take samples of water from water bodies that need to be safe, for example because they serve as sources of drinking water, or places we like to swim, and check for contamination with faeces. However, because faeces contain a lot of organic material similar to non-faecal sources like compost, we need to check for something specific to faeces, which then serves as a *proxy* for faeces. One useful and specific proxy for faeces, and hence faecal contamination, are faecal-specific bacteria called coliforms. These bacteria have the unusual property for microbes found in the environment of using lactose as a carbon source for growth. Lactose is the sugar present in milk and, because humans have been drinking milk for a very long time, some of our intestinal bacteria have learned to use it for growth. So, if we find microbes able to grow with lactose in our sample, it usually means that they are coliforms from faecal material that is contaminating the water sample we are monitoring.

Bromocresol Purple Lactose Broth is a special medium used to grow and detect fecal coliforms, indicators of fecal contamination. Coliforms are the only bacteria that ferment lactose and concomitantly produce high concentrations of acid and gas (mostly carbon dioxide and hydrogen). To carry out the test, we need to have a nutrient medium that allows the bacteria to grow, and a means of detecting acid and gas production. To detect gas production, the culture tube contains a much smaller inverted tube – the Durham vial – which collects the gas, which we can see as a bubble at the top of the tube. To detect the production of acid, the medium contains a so-called acidity (pH) indictor, in our case bromocresol purple, which at normal pH is purple, but at acid pH turns yellow. Thus, if fecal coliforms are present in the sample we take, they will grow in the medium, change its color to yellow, and produce a gas bubble at the top of the Durham vial.

What are its key ingredients?

- Casein peptone and beef extract: food that can be used by most bacteria.
- Lactose: sugar that only some bacteria can ferment.
- Bromocresol purple: pH indicator that becomes yellow at a pH below 5.5 and is purple at any pH above 7.5.

How do we make it?

Bromocresol purple	0,03 g
Lactose	5 g
Casein peptone	7 g
Beef extract	1 g
Water	Make up to 1 liter final volume

- Weigh each of the listed ingredients accurately.
- In a large container, combine the ingredients.
- Pour the distilled water into the mixture while stirring continuously. This helps to dissolve the ingredients.
- Put an inverted Durham vial in a standard glass tube.
- Dispense 10 mL of the medium into each tube.
- Sterilise the tubes by autoclaving at 121°C for 15 minutes. This process kills any unwanted microorganisms and ensures a sterile medium.
- After autoclaving, cool the medium to room temperature.
- Tubes are ready for use.

What do we inoculate it with?
To each tube, we add 1ml of an environmental sample we have collected. Once inoculated, the tubes are incubated overnight at the optimal growth temperature of the bacterium, usually 37°C.

What can we see after incubation?
If there is no growth (no turbidity), we conclude that the water sample does not have any bacteria able to use lactose, casein peptone and/or beef extract as carbon source. If there is growth, the colour of medium turns yellow and the gas is trapped into Durham vial, we can assume that water sample is contaminated with faecal bacteria. If there is growth but the medium did not turn yellow, or there is no gas in the Durham vial, we can discard the possibility of faecal coliforms in water samples.

How do we confirm our interpretation?
Many tests done in biology are not 100% specific, so we often try to confirm results using a second test. We can confirm our observation by adding the same water sample to a confirmative medium termed Brilliant Green Bile Broth, in which the simultaneous presence of bile and brilliant green inhibit almost all Gram-positive organisms and Gram-negative bacteria other than coliforms. If, after 48 hours of growth at 37 °C, we observe turbidity, we can confirm the presence of faecal coliforms in our samples.

How is the interpretation used?
The medium is used in environmental microbiology to detect the presence of fecal contamination in water samples, since pathogens associated with feces can cause gastrointestinal infections in humans. If we detect coliforms in a water sample, it means that the water body from which it came needs attention. For example, a lake or beach area might need a sign warming people not to bathe there; drinking water may need to be boiled or not used. And, of course, action needs to be taken to identify the source of the faecal contamination and stop it.