MicroChat

How and why we grow microbes
Part I: the chemistry and some sorcery called aseptic technique

(Ken Timmis)

Artwork by Joana B. Caldeira, University of Coimbra

So: how do we grow microbes? Almost exactly the same way as we do a chemistry expt.

As in chemistry, we take a flask, mix some chemicals together, for example A and B, and observe them disappearing, and new ones appearing: classical substrates combining to form products, for example X. We represent the chemical reaction as

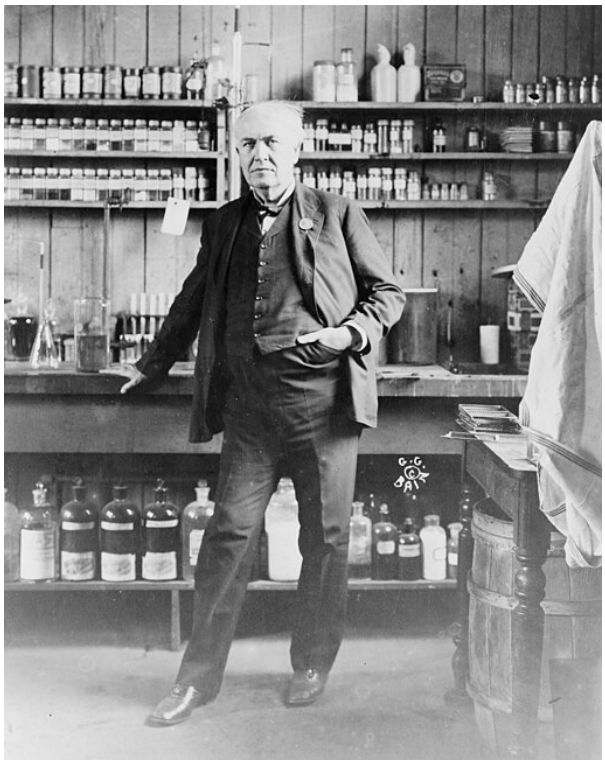
A+B=X.

In microbiology, X = new microbes.

The only real difference is that for chemical reactions we often need extreme conditions like high temperature, high pressure or nasty chemicals, whereas for biological reactions, we don't……except when we do.

Microbes are like us: they feel good at room temperature, and pressure, and don't like acids, or alkalis or nasty solvents…… except when they do (ok: there are things called extremophiles that actually do not like room temperature, etc. In fact, they are stressed out of their tiny minds with conditions we consider to be normal and only feel comfortable when bathing in boiling water, or in strong acids or alkalis….or in oil…or at high pressures. In fact, one line of thought is that

life first kicked off under such conditions and that it took billions of years for us to wean ourselves off extreme kicks.)



https://commons.wikimedia.org/wiki/File:Thomas_Alva_Edison_on_24_February_1911_in_a_chemistry_laboratory,_from-_T.A._Edison_LCCN93500814_(cropped).jpg

But I digress.

Because I now have to confess that there is another little difference between a chemical reaction and growing microbes. This is how we handle the issue of purity of reagents/reactants, which obviously determines the quality of the product, X, which in turn determines the success or failure of the experiment.

In chemistry, everything is simple and straightforward, which is the way chemists like it and why they feel very relaxed doing their stuff. They clean the flask with chromic acid – a very strong acid that cleans very well – and buy the purest reagents they can afford from a chemicals supplier. This ensures that everything proceeds according to plan, and that A + B = X.

However, in contrast to chemists, the purity issue causes microbiologists to be systemically and totally neurotic. The reason is that the reaction carried out is not quite A+B=X. In order to produce microbe X from A+B, we already need a *"little"* bit of X to start with, because X is not a pure chemical but rather a complex mixture of compounds – around 1000 – whose complexity does not allow its creation from pure chemicals alone. In this regard, a rather important difference between chemical X and microbe X is that the microbe is itself able to synthesize most or all of the 1000 compounds it needs to grow, when provided with A and B, and generally some other bits and pieces needed in small quantities, like c, d, e, f and g.

So the microbial growth reaction can be represented as

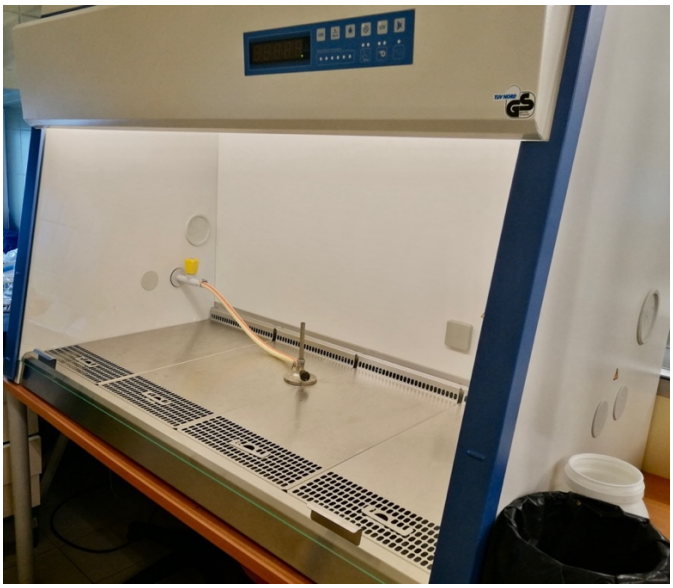
$$A + B + {}_{X} + {}_{c} + {}_{d} + {}_{e} + {}_{f} + {}_{g} = X$$

"Little" is often 1000-10000 cells, but can also be just 1 (especially if it is Y or Z: see below).

The problem is that, unlike chemical reactions in which only A and B are in the flask (or in a closed bottle in a closed cupboard), microbes are all around us, in the air, on our fingers, and especially in the breath we breathe into the flask. And most of these are happy to steal A+B, etc. and grow to produce lots of Y, Z and so on…which, given that we only want X, is a bit of an issue.

So: when we grow microbes, we have to ensure that Y and Z and all the other opportunists crowding around waiting for some A+B are kept at arm's length, and that only X gets into the flask. We do this by a long, excruciatingly boring process we call *aseptic technique*.

Aseptic technique is warfare – the kind we engage in when we put antiseptic cream on a wound to prevent it getting infected. It is all about killing real, potential and even imaginary intruders and using defense barriers to prevent them entering the cosy environment we are creating for X. We work in laminar flow cabinets. They are called this because they have powerful sheets of air flowing upwards at their entrances that act like a barrier to prevent the enemy entering by air. Inside they have flasks of pure alcohol that we use to kill any bugs that might be on surfaces of items we bring into the cabinet, and a bunsen burner for us to burn off any bugs on things we use to introduce X into the flask, before we use them. And when we have finished, we switch on sterilizing ultraviolet lights inside the cabinet to kill off any microbes we may have inadvertently introduced by not paying sufficient attention when a lab mate informs us that it is lunchtime.



A clean bench in which we often carry out manipulations associated with aseptic technique

Aseptic technique is also a state of mind, a sort of mystical state of consciousness, something we feel in our psyches but do not/cannot articulate in words.....all of our senses experience heightened awareness of the slightest danger, like

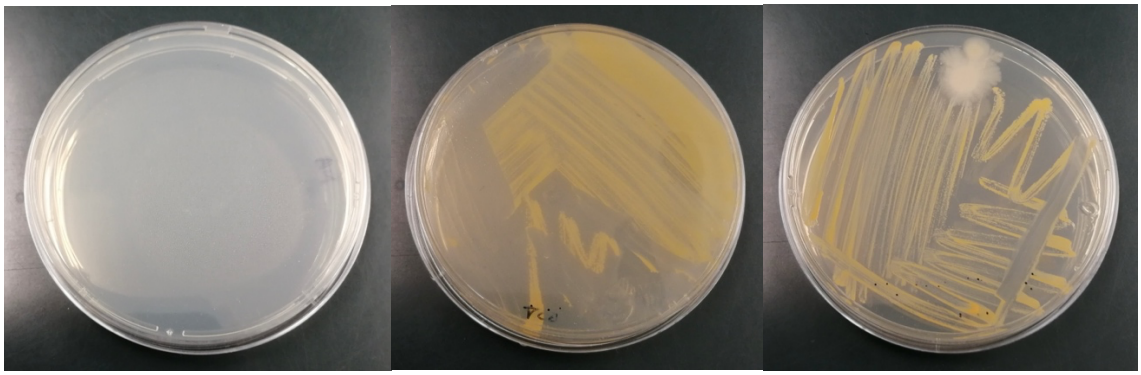
- a draft created at the other end of the laboratory when someone opens a door,
- dropping something in our laminar flow cabinet that might create air turbulence which reduces the efficacy of the laminar flow keeping bugs out,
- a nascent sneeze from a labmate that will, with absolute certainty, ruin our meticulously planned experiment (we detect this even though there may be loud music playing from a radio on the window ledge, or if we are wearing sound-blocking headphones),
- remembering when we open a petri dish that we did not wash our hair this morning (hair harbours a zoo of microbes just waiting to fall from our locks and sabotage microbiology experiments: take a look at the Topic Framework on sloth fur microbiology).

Since time of exposure to dangers of contamination is directly related to the probability of disaster, we are acutely aware of the movement of the second hand of an invisible clock in our

brains inexorably ticking away the seconds. So when we leave freshly poured and steaming agar plates open to cool, solidify and dry (steam leads to condensation, which is a liquid: liquids, like air, represent connectivity, and connectivity is the route that contaminants take to ruin us), we are in a clinical state of stress, wanting to close the plates to prevent air contamination and make them safe, but simultaneously wanting to allow them to dry just a little more, to prevent subsequent contamination via condensation water migrating to the outside of the plate, connecting up with contaminated air, and serving as a trojan horse to let in the enemy.

Artwork by Javier de la Peña Noya, Universidad de Sevilla

Anyway: enough of this complaining about the stresses of being a microbiologist. When everything goes right, and it mostly does for well-trained microbiologists, we are rewarded by an extraordinary biomass yield: our invisible 1000 cells may become increased by a factor of 1 million to reach I billion or more. Microbes are excellent chemists; much better than we are!



Petri dishes containing nutrient agar and incubated overnight at 37°C.
L>R uninoculated; inoculated; inoculated and containing a containing a contaminant.

In Parts II and III, we will discover how we purify X so that it behaves almost like a pure chemical, why we grow microbes in the laboratory, and how we use them.