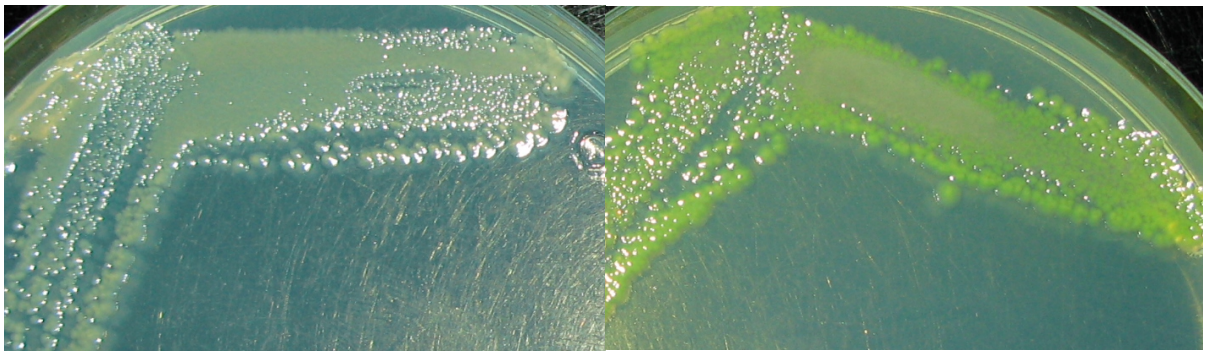
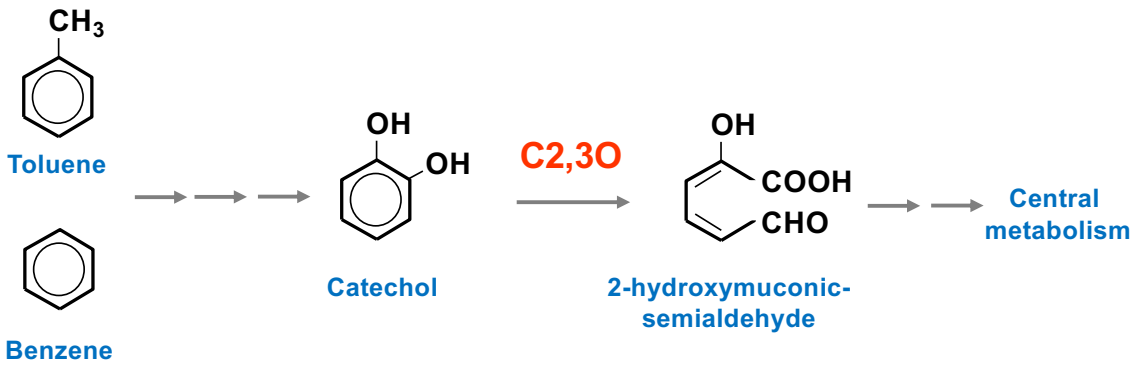
GloriousMicrobialMedia Portrait Gallery: the C2,3O Spray

(Renata Moreno, Luis Yuste, Fernando Rojo)



One very important class of microbes is those that degrade environmental pollutants, because they clean up polluted sites, reduce the environmental impact of their pollutants, and hence protect us against the worst effects of environmental toxins. Such microbes can also be used in biotechnological processes to speed up the natural remediation of polluted sites. These microbes are therefore of high interest to microbiologists and biotechnologists. However, isolating them from the environment may not always be easy, so we often use tricks to achieve this, sometimes using proxies of biodegradation.

One important type of pollutants are the aromatic hydrocarbons, like benzene and toluene, present in crude oil. One of the metabolic pathways employed by microbes to eat such toxic compounds, the so-called *meta*-cleavage pathway, involves the enzyme catechol-2,3-dioxygenase (C2,3O), which converts the metabolic intermediate catechol to a semialdehyde that has a yellow colour.



Scheme of the *meta*-cleavage pathway. Bacteria that contain this metabolic pathway harbour enzymes that can transform some aromatic hydrocarbons such as toluene or benzene into catechol, which is colourless. The catechol-2,3-dioxygenase enzyme (C2,3O in the figure) can cleave the catechol molecule, generating a yellow compound named 2-hydroxymuconic-semialdehyde. This molecule can be further transformed into compounds that enter the central metabolism of the cell. However, if the semialdehyde accumulates in the cells, they become yellow.

Geneticists love coloured compounds because they can often be used for detection and monitoring tests! So, microbiologists use C2,3O as a proxy for microbes able to metabolise toxic aromatic hydrocarbons.

To reveal microbes producing C2,3O, we simply spray a plate of colonies with a colourless solution of catechol in water. Those colonies containing C2,3O, and thus presumptive microbes able to degrade aromatic hydrocarbons, convert the colourless catechol to the yellow semialdehyde and thus turn yellow. The two photos above show colonies of a typical *Escherichia coli* laboratory strain (strain TG1) grown on Petri dishes containing an agar-based growth medium. Cells of colonies on the right contain the gene for the catechol-2,3-dioxygenase enzyme, and turned yellow after being sprayed with catechol, while those on the left remained the original creamy colour.

Like other proteins that produce coloured compounds, the catechol-2,3-dioxygenase enzyme can be used as a reporter to study the behaviour of genes, and also to tag cells to visualize them among a heterogeneous population in which some cells contain the enzyme, and others do not.