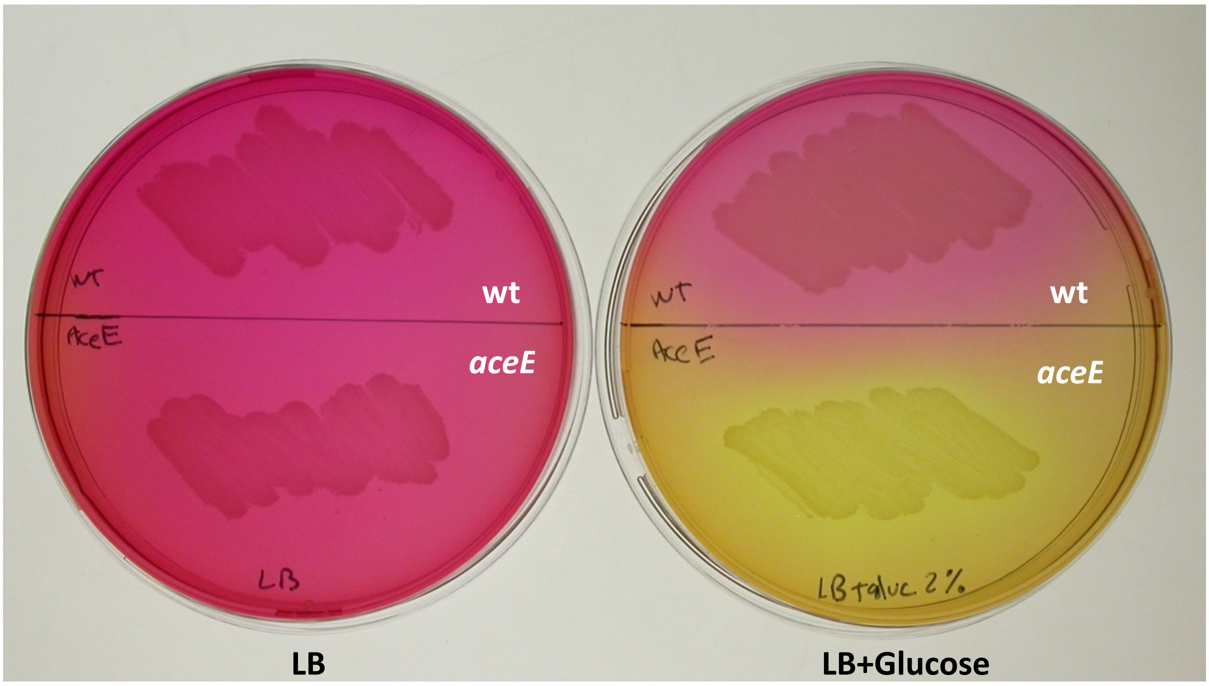
GloriousMicrobialMedia Portrait Gallery: Acidity/Alkalinity Indicator

(Renata Moreno, Luis Yuste, Fernando Rojo)

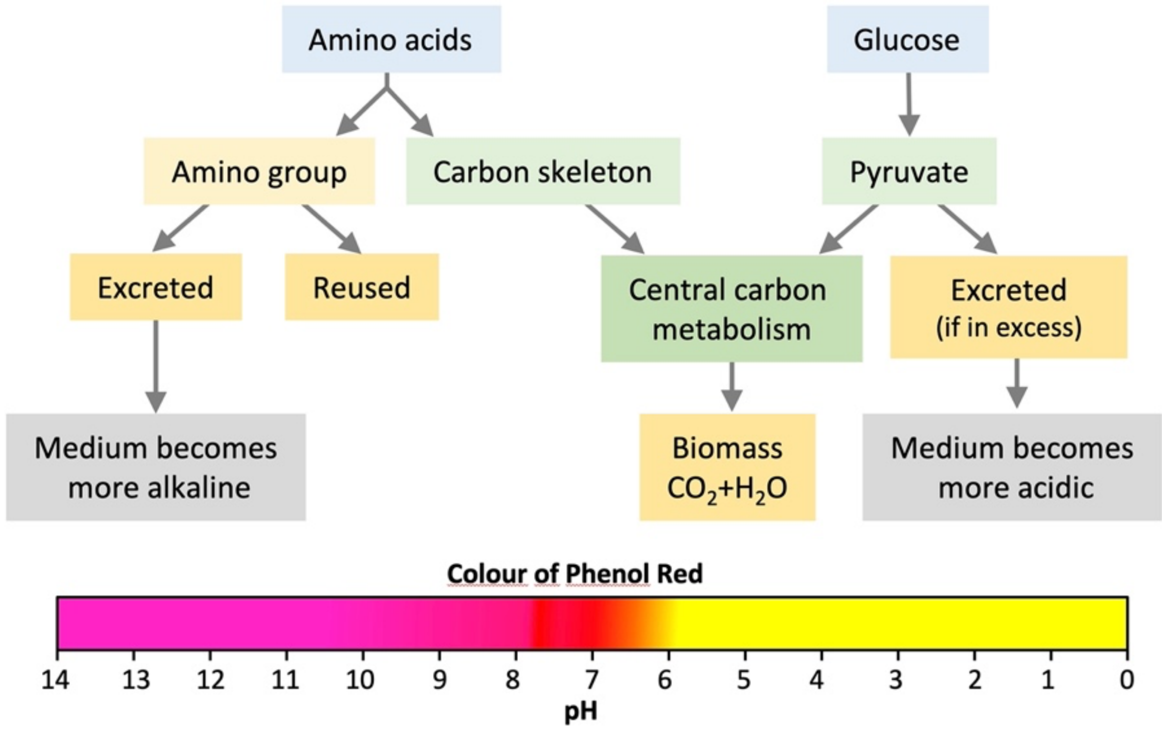


The acidity of the environment is important for bacterial growth. Acidity is measured as a value termed pH, the units of which are expressed on a logarithmic scale from 0 to 14; values below 7 are acidic, and those above 7 are alkaline. Each bacterial species grows best within a certain pH range. The pH value of most natural environments is within the range of 5 to 9, and their microbiota grows best within this range. However, some microbes – so-called extremophiles – prefer very acidic or alkaline environments, and thrive best where the pH is extreme.

The rich LB medium commonly used in research laboratories has a neutral pH, close to 7. Cells growing in LB medium mainly use amino acids as the carbon source, since simple sugars are scarce in it. The metabolism of amino acids results in the production of ammonia, usually in amounts that exceed cell needs. As a result, cells excrete ammonia into the growth medium, making it more alkaline. On the contrary, the metabolism of sugars such as glucose produces pyruvic acid as an intermediate and, if generated in excess, or not further metabolised, it can also be excreted, making the growth medium more acidic.

The plates shown in the photos contain LB medium solidified with agar, and a pH indicator named Phenol Red at a concentration of 0.003%. Phenol Red has a yellow colour when the pH is below 6.8, and a red/fuchsia colour at pH values of 8 or higher. The plate on the right – but not that on the left – was supplemented with glucose.

Both plates were inoculated with a normal *Pseudomonas putida* strain (streaks on top, indicated as wt), or with a mutant strain deleted of its gene coding for one of the subunits of the pyruvate dehydrogenase enzyme (streaks on the bottom, indicated as *aceE*). Both strains can eat the amino acids and turn the medium red. However, whereas the normal strain can also eat glucose completely, and pyruvate is transformed into CO_2 + H_2O so that it does not accumulate, the *aceE* mutant strain metabolizes glucose only to pyruvate, which accumulates and is excreted into the medium, acidifying it. As a result, for both the normal strain and the *aceE* mutant, the medium in the plate lacking glucose is red. However, on the plate containing LB and glucose, growth of the *aceE* mutant strain acidifies the surrounding medium, and the pH indicator turns yellow.



Scheme showing how the assimilation of amino acids and sugars can affect the pH of the growth medium. The metabolism of amino acids results in the production of ammonia, usually in amounts that exceed cell needs. Part of the ammonia is reused to synthesise other compounds containing it, but unused ammonia molecules are excreted to the medium, making it more alkaline. The metabolism of sugars such as glucose produces pyruvic acid as an intermediate and, if generated in excess, or not further metabolised, it can also be excreted to the growth medium, acidifying it. The colour of the Phenol Red as a function of the acidity (pH) is indicated.

Acidity/alkalinity indicators like Phenol Red are very useful colour indicators for detecting/monitoring microbes that change the pH of the medium in which they grow by excreting pH-influencing metabolites.