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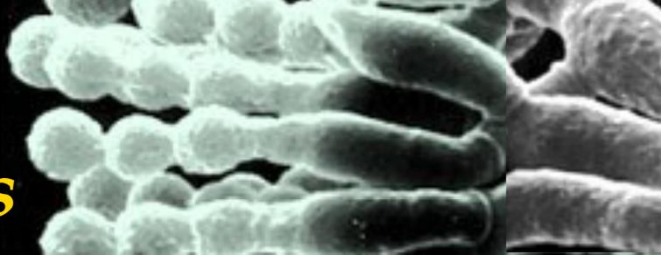
50th Anniversary Articles



Pasteur and Lister through the microscope

John Grainger

Although yet to be known as 'microbiology', the key developments in the subject in the late 1800s came through the need to solve practical problems in fermentation and medicine. The seminal contributions of the contemporaries Louis Pasteur (1822-1895) and Joseph Lister (1827-1912) to advances in these areas are well known but perhaps less well appreciated are the particular contributions that microscopy made to their achievements.

Louis Pasteur

Pasteur published his research on the production of lactic acid from 'sugar', presumably sucrose, by fermentation in 1857. Microscopy provided evidence that the 'organised beings' seen by previous workers were not contaminants in an entirely chemical process but were the agents responsible for that process. He called them 'lactic yeast', a derivation from the descriptions of 'beer yeast' in alcoholic fermentation recorded some 20 years earlier by Theodore Schwann (1810-1882) and others. However, Pasteur noted that the 'tiny globules' that occurred singly or in irregular masses, were much smaller than beer yeast. For this work he observed specimens in a wet mount on a microscope slide, not in a dried smear stained with a dye, an improvement in microscopy not developed until some 20 years later by Robert Koch (1843-1910).

The organisms that Pasteur saw were bacteria which did not move across the microscope's field of view (*i.e*. they were 'non-motile'). They were probably *Streptococcus lactis*, a member of the lactic acid bacteria many of which are still of great importance in making dairy products. In 1860 he published research on alcoholic fermentation by yeasts, recording that fermentation in a medium containing 'sugar' was paralleled by increases in the number of yeast cells seen microscopically and that their mode of growth was by budding.

In 1861 he reported on 'life without air', *i.e*. the first demonstration of the ability of organisms to grow in the absence of oxygen and be killed by its presence; such organisms are now called 'strict anaerobes'. He demonstrated the phenomenon in the production of butyric acid by 'animal infusoria' in which he described the presence of small cylindrical rod-shaped cells about 0.002 mm (2 µm) in diameter and 0.002 – 0.02 mm (2 to 20 µm) in length, sometimes curved, which occurred singly or in short chains, were motile and reproduced by binary fission. Clearly they were not animals, as he believed at the time because of their ability to move, but were bacteria, later thought to be of the genus *Butyrivibrio* which also occurs with other butyric acid-producing bacteria in the rumen where butyric acid produced from fermentation of cellulose is an important component of the diet of cattle and other ruminants.

Pasteur then investigated alcoholic fermentation and described for the first time the ability of yeasts to grow both in the presence of oxygen and in its absence. He also noted the different efficiencies of the two processes as regards the rates of the growth of yeast and

utilisation of 'sugar'. Microbes that are able to grow in the presence and absence of oxygen are now called 'facultative anaerobes'.

The applications of Pasteur's observational skills in practice are well illustrated by his visit to the Whitbread brewery in London in 1871. Using his own microscope, because the brewery did not possess one, he saw among the yeast an abundance of 'disease filaments' in the outflow of some fermentation vats that were suspected of showing signs of spoilage. He counted the numbers of contaminants and made drawings for the benefit of the Head Brewer, and also noted their presence in the deposit in some other casks after not finding any in the liquid above the deposit (a lesson for anyone learning to observe specimens microscopically!). On returning to the brewery a few days later, Pasteur found that a microscope had been acquired and new samples of yeast had been obtained (**Figure 1**).

Figure 1. A late 19th century microscope similar to one used by Pasteur.

Joseph Lister

The first method for isolating micro-organisms in pure culture was devised in 1875 by Oscar Brefeld (1839-1925) working with fungi. The same advance with bacteria was first achieved in 1878 by Lister who, stimulated by Pasteur's observations on the presence of micro-organisms in the air, had published his work on antiseptics in surgery in 1867. Lister then sought to establish the importance of using pure cultures for studies in pathology turning again to Pasteur for inspiration, this time to his work on the lactic fermentation. Through choosing to study the souring of milk, Lister found a ready source of bacteria and a convenient experimental system (**Figure 2**) with a sterile liqueur glass (A) and lid (B) beneath a glass dome (C) placed on a glass plate (D).

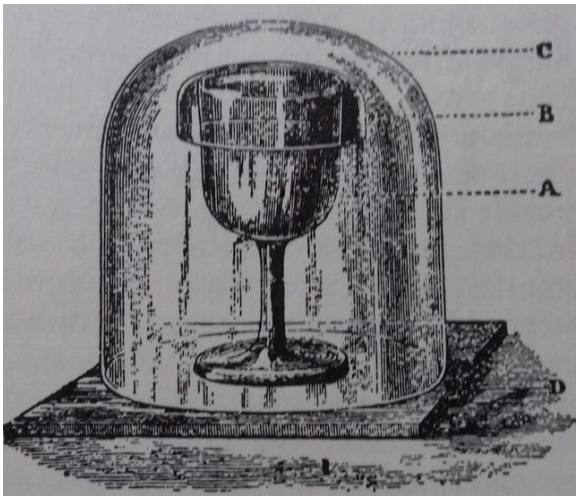


Figure 2. Lister's equipment for isolating pure cultures of bacteria (from *Transactions of the Pathological Society of London*)

He associated the coagulation and souring of milk with the presence of a specific bacterium which he described as non-motile, oval cells 1/20,000 inches (about 1.3µm) in dimension, occurring in pairs and chains of 3, 4 or more cells and, crucially, noted that they were the only microscopical form present in the soured product. He named them *Bacterium lactis.*

Lister's procedure was to dilute a sample of sour milk until microscopic examination of *several* separate specimens revealed the presence of on average only a single bacterium in a field of view. He saw the need to examine several specimens because he was the first to realise that the viewable volume of one specimen was too small to be certain that

only one type of organism was present in the whole sample, *i.e*. it was a pure culture. By showing that the milk had been caused to sour by the action of a pure culture, he confirmed Pasteur's conclusion that bacteria are responsible for the lactic fermentation.

Lister's method for obtaining pure cultures had disadvantages as it was cumbersome and only suitable for making a pure culture of the predominant organism in a mixed population. It was soon overtaken for routine use in 1881 by the simpler and more direct use of solid surfaces introduced by Koch. Nevertheless Lister had created the first method for obtaining bacteria in pure culture and had illustrated the principle that a particular bacterium is responsible for a specific disease. Furthermore, he used microscopy to estimate the number of bacteria present per ml of the soured milk by knowing the dilution of the sample, measuring the volume of diluted sample added to the microscope slide, counting the number of cells in a field of view and measuring the area of the field of view. Nowadays his dilution principle is the basis for the Most Probable Number Method for estimating the number of microbes in a liquid sample which is still used in the bacteriological examination of drinking water supplies.

It has been argued that perhaps for more than most other sciences, early developments in microbiology depended on improvements in microscopy because in the late years of the 19th century the resolving power of microscopes left much to be desired. It is interesting to consider how differently the subject might have developed had the limits of microscopy not been improved by Ernst Abbe (1840-1905) who introduced the oil-immersion lens and an effective condenser and by Koch's contributions (see article: Bacterial endospores: their roles in resolving the spontaneous generation controversy and understanding cell development).

AUTHOR PROFILE

John Grainger gained a BSc in Bacteriology at the University of Birmingham and a PhD in Microbiology at the University of Reading where he made his academic career becoming Head of the Department of Microbiology and then a Visiting Research Fellow. Alongside research interests in environmental microbiology he developed a parallel career promoting microbiology and biotechnology in schools, mainly through MiSAC of which he is Chairman and as Founder and former Director of the National Centre for Biotechnology Education. Work overseas included membership of the management board of the European Initiative for Biotechnology Education. He is a Chartered Biologist, a Fellow of the Royal Society of Biology (RSB) and has awards from the RSB and the Microbiology Society.