

## **The *Bacillus* Cell Factory Innovation and European Regulatory Framework.**

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Dr. Colin Harwood, Emeritus Professor of Molecular Microbiology at Newcastle University, directed HMC consultancy and has amassed over 50 years of research experience with more than 150 publications, primarily focusing on the genetics and molecular biology of *Bacillus* bacteria. His work, notably impacting biomanufacturing, concentrated on protein secretion and responses to environmental stress. Harwood played pivotal roles in numerous EU-funded research projects and collaborated extensively with biotech companies worldwide. He also served on the executive boards of significant organizations such as the European Federation of Biotechnology (EFB) and the Federation of Microbiology Societies (FEMS), making substantial contributions through consultancy, including for the EFSA. Recognized as a distinguished lecturer, he has been invited to speak at international conferences globally.

The exploitation of microbes and their enzymes goes back many centuries, with fermented food products such as wine, nattō, yoghurt and bread making being prominent examples. In 1922, the  $\alpha$ -amylase Rapidase, produced by a strain of *Bacillus*, revolutionized the industrial conversion of starch into sugar. In 1959, the alkaline/serine protease, Subtilisin, isolated from *Bacillus subtilis*<sup>1</sup>, was the first enzyme to be added to a commercial detergent powder. However, the recognition, in the late 1960s, that enzymes could be engineered to improve their native characteristics was a gamechanger that led to the development of a highly innovative global industrial biotechnology industry. This industry offers environmentally compatible alternatives to traditional global warming and polluting oil-based technologies. Nowadays, microbes are exploited as pre- and pro-biotics, for environmental remediation and as hosts for the production of metabolites, proteins and enzymes. I will use the *Bacillus* cell factory to provide examples of each of these aspects of biotechnology, and the regulatory framework in Europe under which their use is controlled.

The application of enzyme engineering was first applied to Subtilisin. Pioneering work by Alan Fersht and colleagues demonstrated how this enzyme could be engineered to be more tolerant to detergents, oxidation and temperature. This was followed by extensive selective screening programmes aimed at isolating bacteria and fungi able to produce enzymes with a wide range of novel catalytic and physiological characteristics. For many decades these enzymes have provided the raw material for a wide range of industrial catalytic processes. Alongside the need to expand the range of enzymes was the complementary need to produce the enzymes in the most cost-effective manner. In some cases, the original enzyme hosts were sufficiently malleable to act as the producer strain, but more often it was necessary to develop industrial host strains capable of producing a wide range of enzymes from disparate sources.

*Bacillus* species, probably the most widely used for commercial enzyme production, provide a model for the development of generic hosts. I will briefly outline the main factors that influence productivity, stressing the role of protein folding as a significant factor affecting yields. I will also discuss how signal peptide screening, quality control processes and folding factors have been shown to be important for increasing production yields.

Bacteria have evolved over 3 billion years, and despite being regarded as simple organism, they have complex regulatory networks that allow them to respond to the multifactorial environmental and nutrient stresses encountered in their natural habitats. Many of the pathways involved in these survival mechanisms are not necessary required for growth in bioreactors. This was first recognized in Japan and, as a result, a number of genome minimalization projects were initiated. These are still being continued in Europe, with some success in terms of increased productivity for certain products. However, the remnants of the cell's regulatory networks can be disruptive, making predictions about key factors such as growth rate and yield difficult.

The use bacterial strains for commercial purposes in the EU is controlled by the European Food Safety Authority (EFSA), who ensure compliance with EU regulations. I will discuss aspects of this regulatory framework within which biotechnology companies must operate. This includes precise strain identification and the categorizing of strains as having Qualified Presumption of Safety (QPS). The qualifications include the absence of certain antibiotic resistance and toxin genes, and the production antimicrobial peptides. Traditionally enzymes have been extracted from industrial bioreactors for use in specific processes. However, increasingly, whole organisms are being developed as environmentally compatible approaches to improve animal and plant health. Often such strains have innate resistance genes that would, if not removed, prevent their release. In Europe this has led to concerns about the release of genetically modified organisms (GMOs) into the environment. Whilst genome management technologies such as CRISPR can undertake precise genome manipulations, their use, even for the removal of potentially hazardous genes, is proscribed in the EU.

Finally, the industrial enzyme market is currently undergoing a step-change revolution following advances in Artificial Intelligence (AI). Fundamental to these changes is whole genome sequencing technology and the resulting genome databases that can be mined for enzymes with novel catalytic properties. This resource can be combined with protein structure prediction software such as AlphaFold, based on the Google DeepMind AI system. This allows enzyme/substrate interactions to be modelled with a high degree of certainty without the need for de novo structural studies. High throughput algorithms facilitate the mining of protein databases to reveal enzymes/substrate interactions which, following the application of enzyme engineering principles, can lead to the design of bespoke enzymes for novel catalysis.

## Reference

<sup>1</sup>Bremner et al. (2023). A model industrial workhorse: *Bacillus subtilis* strain 168 and its genome after a quarter of a century.

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