Early Biotechnology: The Delft Connection

Early Dutch microbiologists fostered their own golden age of microbiology

J. W. Bennett and Herman J. Phaff

Delft, a small city in The Netherlands between Rotterdam and The Hague, is best known for its distinctive blue and white porcelain and as the birthplace of the painter Jan Vermeer. But it is also a special place in the history of microbiology. For example, Antonie van Leeuwenhoek (1632–1723), the "Father of Microbiology," lived in Delft and is buried in a place of honor in the Oude Kerk (Old Church). Less well known are two other microbiologists, Martinus Willem Beijerinck (185 l-193 1) and Albert Jan Kluyver (1888–1956). In succession, these men held the chair of general and applied microbiology in the Department of Chemical Technology of the Technological University at Delft, making the "Delft School" famous and creating a remarkable scientific tradition. It can be argued that both general microbiology and classical biotechnology came of age in Delft.

An embossed plaque honoring van Leeuwenhoek hangs on a gate near the entrance to the Oude Kerk in Delft

Beijerinck—One of the Big Four in Bacteriology

Martinus Willem Beijerinck was called "one of the big four in bacteriology" (along with Winogradsky, Pasteur, and Koch) by Grainger in his *Guide to the History of Bacteriology*. However, in the early years of his career, there were few signs that Beijerinck would be attracted to microbiology.

Beijerinck did his doctoral studies at Leiden University on the morphology of plant galls. This early botanical research displayed the high standards of execution and the unusual interests that were to characterize Beijerinck's research throughout his life. While teaching at the Agricultural University (Hoogeschool) in Wageningen, he wrote not only on plant galls, but on phyllotaxis, the formation of adventitious organs, and gumming disease in fruit trees. His work came to the attention of Hugo de Vries (one of the rediscoverers of Mendel), and in 1883, just 7 years after receiving his Ph.D., Beijerinck was elected to the Royal Academy of Sciences in Amsterdam.

In Delft, the director of the Nederlandsche Gist-en Spiritus fabriek ("Netherlands Yeast and Alcohol Works," now Gist-Brocades) was an enlightened industrialist named J. C. van Marken. A graduate of the Technological University in Delft, his company specialized in the production of baker's yeast and alcohol. van Marken, who believed that the success of his company depended on basic research, sought a strong talent to lead the laboratory he planned to establish. In the fall of 1884, he offered Beijerinck that position, at the then-high salary of f. 4,500, which was over twice what Beijerinck was earning as a teacher.

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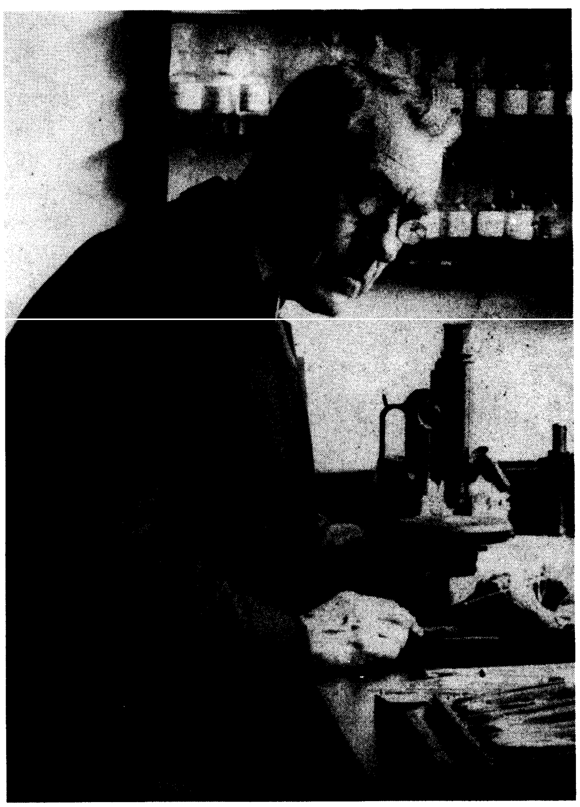
Beijerinck accepted the offer on 1 January 1885 and started work in Delft the following September. During the intervening months he visited the mycologist De Bary in Strasbourg, France, and the yeast pioneer Hansen in Copenhagen, Denmark. He had also planned to visit Koch, but according to L. E. den Dooren de Jong, one of his biographers, he fought with De Bary, claimed Hansen had fobbed him off with trifles, and "since he expected to learn even less from Koch," cancelled his proposed visit to Berlin. Beijerinck was a self-taught microbiologist with a difficult personality.

At the Nederlandsche Gist-en Spiritus fabriek, Beijerinck was charged with studying the basic properties of yeasts and the microbial contaminants that interfere-with the scaleup of alcohol production. With characteristic flexibility, he worked on butyl alcohol fermentation, isolated and named the enzyme lactase, characterized the microbial flora of kefir, and was involved in calming rumors that compressed yeast could act as a carrier of cholera germs. Simultaneously, "in his spare time," he isolated the first pure cultures of nitrogen-fixing bacteria from root nodules, applied the experimental approaches of bacteriology to green algae and gonidia of lichens, and produced a number of fundamental papers on the physiology of luminescent bacteria. (Nowadays, according to an employee of Gist-Brocades, management is not "quite so tolerant" of such wide-ranging research.)



Beijerinck in his laboratory at the Technological University, Delft

Beijerinck's remarkable achievements attracted attention, and he was offered several positions outside Delft. van Marken, motivated perhaps by a desire to keep this genius close by, was influential in creating the chair of general and applied microbiology in the Polytechnical University in Delft. In 1895, Beijerinck became a professor and remained at the university there until his retirement in 1921.

During his years at the Polytechnical University (a name later changed to Technische Hoogeschool or Technological University, at his insistence), Beijerinck continued his eclectic microbial studies. In 1898 he published results showing that the causative agent of tobacco mosaic virus (TMV) is filterable, diffusible, and precipitable and that it multiplies only in living tissue. He called the infective principle a "contagium vivum fluidum" or virus. When informed that Iwanowski had already established the filterable nature of TMV 7 years previously, Beijerinck was quick to acknowledge Iwanowski's work. Although Iwanowski published earlier, he tried to prove the bacterial nature of the filterable agent. By contrast, Beijerinck clearly understood that he was dealing with a different form of life and predicted that other plant diseases might also be caused by similar "contagium fluidum." His insights founded virology.

Beijerinck contributed other key findings during his years at the Technological University, many stemming from his systematic application of enrichment cultures, which opened a rational approach to microbial ecology. He introduced the genera *Lactobacillus, Aerobacter,* and *Acetobacter* ; did basic work on sulfate reduction and denitrification; and isolated and described the first free-living nitrogen-fixing bacteria. Far ahead of his time, he advocated the advantages of microbes as model systems in genetics, publishing a paper in 1917 entitled "The enzyme theory of heredity" in which he proposed (albeit opaquely) that every hereditary characteristic of an organism "corresponds to one or more enzymes."

Beijerinck was forced to retire in 1921, on his 70th birthday. He was so bitter about having to leave his laboratory with attached home that he never returned to Delft. Living in retirement at his country home in Gorssel, however, he received many visitors, including Selman A. Waksman in 1924.

Kluyver, Although Vastly Different, Continues the Tradition

Meanwhile, at the Technological University in Delft, a young, nearly unknown botanist-microbiologist named Albert Jan Kluyver was appointed as Beijerinck's successor. Personally, the two men were extraordinarily different. Beijerinck was notorious for his stern manner; his contempt for beer, tobacco, and women; and his uncompromising temperament. Kluyver was happily married, smoked incessantly, had an outgoing, charismatic personality, and knew how to relax over a drink with his students. However, both men were prodigious workers with extraordinary

At the 1954 ASM President's Reception, A. J. Kluyver (right) congratulates his former student C. B. van Niel (left)

intellects, and they both appreciated the far-reaching implications of specific experimental findings.

Kluyver's first task as a new professor was to overcome the "scientific snobbery" of-the assistants he inherited from Beijerinck, whose expectations of him "were not very high." Kluyver reorganized the laboratory and acquired modern equipment. Connections were resumed with van Marken at the Nederlandsche Gist en Spiritus fabriek. Students, who were scarce in Beijerinck's time, soon flocked to the reorganized laboratory.

C. B. van Niel Was among Kluyver's Early Students

One of the first students to sign on was a young man named C. B. van Niel. After obtaining a degree in chemical engineering, he accepted a position as assistant to Kluyver, where he cared for a collection of bacteria, yeasts, and fungi; assisted undergraduates; and prepared demonstrations for Kluyver's two lecture courses& general and applied microbiology. Although van Niel soon developed a deep interest in the purple bacteria and in isolating pure cultures of several genera, his doctoral research was on propionic acid bacteria which he isolated from Swiss cheese.

van Niel published his dissertation in 1928, and that same year he was offered an associate professorship at the new Jacques Loeb Laboratory at the Hopkins Marine Station of Stanford University on the Monterey Peninsula of California, where he stayed until his retirement in 1962. van Niel was elected president of ASM in 1954.

(One of us [H.J.P.] joined Kluyver's laboratory in 1935 to study pectolytic enzyme secretion by a fungus, and obtained a degree in chemical engineering in 1938. Kluyver's enthusiasm for the U.S. approach to scientific problems induced me to continue my education at the University of California in Berkeley, where I completed a Ph.D., subsequently obtained a faculty position, and eventually transferred to the University of California, Davis. In 1954, Kluyver paid a visit to our campus.)

Doctoral students in Kluyver's laboratory worked on an astounding diversity of topics, including methane fermentation, nitrate reducing bacteria, propionic acid bacteria, *Spirillum* spp., beer spoilage, sarcinas, microbial amylases, bacterial treatment of latex to produce more uniformly vulcanizing natural rubber, the introduction of the submerged culture technique for growing molds (ultimately leading to its application for the industrial production of riboflavin, penicillin, and other antibiotics), and a series of monographs on the ascogenous and asporogenous yeasts. Kluyver typically brought flasks of luminescent bacteria to his lectures on the subject, darkening the room to illuminate their striking metabolic properties.

The yeast monographs comprise an exhaustive survey of that field and describe the extensive collection of yeast cultures maintained by the Delft laboratory in collaboration with the Centraalbureau voor Schimmelcultures. These works mark the beginnings of the Delft School of yeast taxonomy.

Kluyver was not an experimentalist in the sense that he pursued research projects on his own. However, through his extensive reading and vast knowledge of the microbiological and biochemical literature, he stimulated and guided his numerous students and postdoctoral visitors.

Kluyver's contributions to microbiology are so basic, so diffuse, and so well enmeshed in current theory and practice that they are harder to eulogize than Beijerinck's. Kluyver was the father of comparative biochemistry; he wrote extensively on the unity of biochemistry; he promulgated the use of microbes for elucidating biochemical pathways and energy transformations and as aids in biochemical technology. He travelled widely, delivering elegant, philosophical lectures on broad microbiological themes. Some titles from his lectures and reviews, reprinted as appendices in the 1954 biography edited by A. F. Kamp et al., illustrate the breadth of his interests: "Life's fringes,"

A. J. Kluyver (right) and H. J. Phaff (left) in 1954 at the latter's home in Davis, Calif.

"Microbial metabolism and its industrial implications," "Microbes and life," "Homo militans." It was Kluyver who first said, "From elephant to butyric acid bacterium-it is all the same!" (A similar epigram is usually attributed to Jacques Monod in speaking of *Escherichia coli*).

In writing about the "The Delft School" for an American audience in 1949, C. B. van Niel noted:

> It would be foolish to insist that the principles of comparative biochemistry would not have been developed if it had not been for Kluyver's penetrating approach, just as it would be foolish to contend that microorganisms would not have been discovered if Antonie van Leeuwenhoek had not done so . . . . Nevertheless, the familiarity with the vast diversity of the conditions under which life can exist and manifest itself, especially in the world of microorganisms, made available for Kluyver's scientific contemplation an immensely greater range of patterns than that presented by the higher plants and animals. And the result was the enunciation of the most far-reaching generalization.

Most textbooks describe the golden age of microbiology in terms of clinical science, using the germ theory of disease and Koch's postulates as examples. However, simultaneously, the foundations for microbial ecology, classical biotechnology, and of general microbiology were being established in centers such as Delft. Beijerinck's and Kluyver's far-reaching contributions can be better appreciated now than during their lifetimes. ☐

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