Sergei Winogradsky: Founder of Soil Microbiology

King-Thom Chung and Christine L. Case

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The establishment of microbiology as a biological science is largely credited to Louis Pasteur, Robert Koch, Martinus Beijerinck, and Sergei Winogradsky. **Sergei Nikolaevitch Winogradsky** was drawn to microbiology because he was "impressed by the incomparable glitter of Pasteur's discoveries." In 2001, there is a renewed interest in the work of Winogradsky due to recognition of the role of microbial ecosystems in bioremediation and agriculture. Winogradsky's techniques led to the isolation of new and useful bacteria and his *enrichment culture technique* is now a staple in environmental microbiology. Born and educated in Russia, much of Winogradsky's scientific work was done outside of his native land. We will see how political changes can affect scientific endeavors as this scientist responded to the vicissitudes of life in turbulent periods.

Winogradsky discovered
Beggiatoa minima
Clostridium pasteurianum
Cytophaga hutchinsonii
Nitrosococcus nitrosus
Nitrosocystis javaensis
N. coccoides
Nitrosomonas europaea
Nitrosospira briensis
N. antarctica
Nitrobacter

Winogradsky was born on September 1, 1856 in Kiev, Russia. He spent a privileged childhood playing in this family's large gardens and attended by servants. At 10 years of age, the young Sergei entered the Second Gymnasium. His father chose the Second Gymnasium because it offered both Greek and Latin. At that time, educated people felt that classical languages were a sound basis for education. Sergei found the classics "not only uninteresting and unpleasant, but depressing, both physically and mentally." In 1873, Sergei began studying law at the University of Kiev as had his father and brother. He found the law uninteresting and transferred to the division of natural science. However, this was also boring and Sergei stopped attending classes. Subsequently, he studied music but this was “without any activity of the brain.” In 1877, Winogradsky quit music and returned to the natural sciences at the University of St. Petersburg. Here, he had the opportunity to study under excellent scientists and, from them, he began to appreciate science. In 1879, he married Zinaida Tichotzkaia; they remained married for 60 years and had four daughters. In 1881, Winogradsky graduated with a diploma in science and became a candidate for a professorship at the University. He completed his post-graduate studies in plant physiology studying the effects of organic and inorganic nutrients on the wine yeast, *Mycoderma vini*.

The Doctrine of Monomorphism

In 1885, Winogradsky accepted a position at the University of Strasbourg, Botanical Institute under Anton de Bary, where he began his investigations on *Beggiatoa*. *Beggiatoa* was surprisingly well known at the time considering that it had never been grown in pure culture. Researchers knew about *Beggiatoa* because it formed large mats in sulfurous waters and, because of its large size (9-µm cells forming filaments up to 120 µm), it was easily visible with a light microscope. *Beggiatoa* would not grow on the conventional nutrient media developed by Koch and his colleagues, consequently researchers only saw mixed cultures in the samples they viewed in the microscope. These mixed cultures provided arguments for the proponents of pleomorphism—the notion that bacteria change shape. Winogradsky developed a method of culturing (enriching for) *Beggiatoa* by imitating its natural environment on glass slides and silica gel and he observed that the cells were rods and not pleomorphic. His observations affirmed the doctrine of monomorphism. He later wrote "bacteria are…like other organisms, characterized by morphological types that can and should be systemically grouped into genera and species."

Chemoautotrophs

At the time, *Beggiatoa's* intracellular sulfur granules were believed to be formed by the bacteria reducing H_2S. Winogradsky proved that *Beggiatoa* used H_2S; it did not form H_2S. He showed that the organism oxidized H_2S to elemental sulfur (S^0) that was stored in the cells and could be further oxidized to sulfuric acid (SO_4^{2-}). He further showed that the sulfur granules released from dead cells formed the H_2S other researchers thought the bacteria made. While learning to grow Beggiatoa, Winogradsky found that it would not grow in the presence of organic compounds. In 1887, Winogradsky wrote "I have shown that the reason for this is that these organisms have a nutrition which is different from all other non-chlorophyll containing organisms." Winogradsky proved that the sulfur granules resulted from the oxidation of H_2S for energy and that the bacteria used CO_2 as a carbon source. This work established the existence of chemoautotrophs which Winogradsky called "anorgoxydants."

Nitrogen oxidation

After de Bary's death in 1887, Winogradsky settled in Zurich, working at the University of Zurich. His investigations were concentrated on the oxidation of nitrogen in the soil. He proved that the oxidation was a two step process: $NH_3^+ \rightarrow NO_3^-$ and $NO_3^- \rightarrow NO_2^-$ and that each step was performed by different organisms.

Winogradsky's interest in nitrification continued throughout his career and he isolated several genera of nitrifying bacteria. The type species for the genus *Nitrobacter*, *N. winogradskyi* (ATCC 24391) was named for Sergei Winograsky.

Years later, Winogradsky's daughter Helen would work on nitrogen-oxidizing bacteria at the Pasteur Institute. She isolated and described the new genera *Nitrosogloea* and *Nitrosocystis*; and in 1933, coauthored a paper with her father on *Nitrosospira*.

Clostridium pasteurianum

Winogradsky took up the problem of nonsymbiotic nitrogen fixation. He inoculated liquid carbohydrate, nitrogen-free media with soil. After a pellicle of aerobic bacteria grew on the surface, he discovered and isolated a nitrogen-fixing obligate anaerobe growing below the surface. Winogradsky identified the organism as Clostridium pasteurianum. The pellicle-forming aerobe was also capable of fixing nitrogen. Beijerinck later isolated and identified it as *Azotobacter chroococcum*.

Retting

In 1895, Winogradsky took on the problem of retting flax and hemp. In Russia, retting was done by stacking plants in a stream and covering the plants with soil for four to six weeks, until the plant fibers were loosened (because the intercellular pectin was gone). This process added a large organic load to streams increasing the biochemical oxygen demand. Winogradsky showed that retting could be done with pure cultures of anaerobic bacteria, away from a stream. Winogradsky's work became know world-wide and Louis Pasteur offered him a position at the new Pasteur Institute, however Winogradsky preferred to establish microbiology in his homeland, Russia.

Altruism

Prince Oldenburg placed Winogradsky in charge of defenses against Asiatic plague. He and his colleagues were forced to work away from his laboratory in the fort of Alexander I. Although laborious, the work on plague was successful, but the Minister of Finance, an enemy of Prince Oldenburg, attacked the scientists' work. Through the personal efforts of Winogradsky, these unfair criticisms were deflected, but he remembered this “as a typical example of my relations with the ruling clique of the Russian Empire.”

In 1902, Winogradsky was appointed director of the Institute of Experimental Medicine and editor of the journal, *Archives of Science*. The journal was printed in both Russian and French, and under Winogradsky’s leadership, it became the foremost scientific journal in Russia and well known throughout Europe. He donated his entire salary from the Institute for construction of a bacteriological laboratory. The money was eventually used to build the Library of the Institute of Experimental Medicine.

The Russo-Japanese War took money away from scientific research and education thereby preventing Winogradsky from reorganizing the Institute and fostering the growth of microbiology in Russia. Winogradsky resigned his post in 1905. The Institute moved almost entirely in the direction of human medicine—an area that held no interest for Winogradsky. He had always felt that cultivated bacteria “draw importance to themselves,” whereas those whose growth requirements are more complex and elusive “escape attention.” In 1912, Winogradsky left the Institute and settled on the estates in the Ukraine left to him by his father. During World War I, he supplied horses and farm produce for the Russian Army.

Iron Bacteria

Following the Bolshevik revolution of 1917, old, wealthy families were forced to leave Russia–Winogradsky and his family among them. Due to poor health, his wife remained in Kiev. Winogradsky returned to his villa in Zurich. He tried repeatedly and unsuccessfully to contact his wife in Kiev. He joined other Russian professors in the new country of Yugoslavia, accepting a professorship at the Agricultural Institute of the University of Belgrade. From Belgrade, he hired smugglers to bring his wife out of Russia. He feared she might not be able to stand the 200-kilometer wagon trip, happily she was able to join him.

The Institute at Belgrade had neither laboratory nor library. He did, however, find a copy of *Centralblatt für Bakteriolge*, from which he could learn about discoveries during the past 17 years. To his surprise, he found that very little had been done with the autotrophic bacteria. He immediately prepared a paper, “Iron Bacteria as Inorganic Oxidants” which was accepted and published in the *Centralblatt* in 1922. Winogradsky used the same techniques that he had used with *Beggiatoa* to show that bacteria could oxidize Fe^{2+} to Fe^{3+}. The paper attracted immediate attention and in February 1922, Emil Roux, head of the Pasteur Institute, invited Winogradsky to organize a division of agricultural bacteriology at the Pasteur Institute. This time, Winogradsky accepted the invitation to join the Pasteur Institute.

Nitrogen fixation

He set up the new agricultural bacteriology division in Brie-Comte-Robert and started studying bacterial complexes in soil, especially those involved in the nitrogen cycle. Winogradsky was aware that the complexity of soil bacteria required studying them in their natural habitats as well as in cultures. His concept was that in the soil there was competition of nutrients and that a majority of organisms are in a dormant state. He cited the example of the *Azotobacter*, which was active in isolation, but remained "obstinately at rest in the midst of the soil.” But, the use of laboratory methods exclusively, e.g., isolation, pure culture, and bacteriological media, could not establish *real* soil science. He believed that soil microbiology is an independent science that should be carried out under conditions as near nature as possible, "in the soil itself."

While at Brie, he demonstrated that bacteria in root nodules, not the plants, were the active agents for nitrogen fixation. This work aided the development of commercial cultures of nitrogen-fixing bacteria by Ira Baldwin at the University of Wisconsin.

Cellulose Decomposition

Selman Waksman speculated that while walking through his forests and fields in the Ukraine between 1905 and 1917, Winogradsky must have wondered about the disappearance of cellulose—which led to his research on cellulose decomposition. Between 1926 and 1929, Winogradsky embarked on a detailed study of aerobic organisms involved in cellulose decomposition. He recognized the existence of several bacterial genera capable of attacking cellulose and that these bacteria synthesize polyuronides found in soil. He was the first person to describe the fusiform, cellulose-degrading cells in the genus *Cytophaga*.

Retirement

Winogradsky's wife died in 1939 and Winogradsky retired in 1940 after the German invasion of France. Nazis had commandeered his home in Brie several times and Winogradsky found it necessary to physically remain in his home to prevent looting. German occupation of Poland cut off contact with his daughter, Katherine. Waksman, who attempted to keep in contact with Winogradsky, said "a black curtain descended upon Winogradsky" during the German occupation.

During his retirement, Winogradsky assembled and translated all his papers into French in a 900-page tome entitled "Half a Century of Microbiology Research," which the Pasteur Institute had promised to publish. At the end of World War II, there was not enough paper or funding in Europe for publication. Fortunately, the U.S. National Academy of Sciences donated the paper and the Rockefeller Foundation and Rutgers University funded the project; the book was published in 1950. Sergei Winogradsky died on February 24, 1953, at the age of 97.

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