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Microbes, Fermentation and Industrial Microbiology:

From Antiquity till the early 1900s

July/August/September 2021 v.71 n.3

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Letter from the Editor-in-Chief

It was wonderful to see so many of you at the 2021 SIMB Annual Meeting in Austin, Texas! Congratulations to Adam Guss for putting together an exciting and informative program. It was also so good to be able to interact in person again though attendance at the meeting was somewhat reduced. I certainly hope that the COVID-19 pandemic will be better under control next year so the upcoming 2022 Annual Meeting in San Francisco, California, will have great attendance and participation!

Usually, scientific meetings are an opportunity for participants to focus on the latest advancements in their fields. During the 2021 SIMB Annual Meeting, this was certainly true. Furthermore, events occurring beyond the walls of the meeting venue do not immediately impact the science that is being presented and discussed. This year's meeting was different in this way. Obviously, the pandemic impacted all aspects of the meeting, from the overall logistics to the discussions being held in the conference rooms. However, while the meeting was in progress, the Working Group I of the Intergovernmental Panel on Climate Change (IPCC) issued their Sixth Assessment Report, Climate Change 2021: the Physical Science Basis on August 9th. This report definitively states that climate change is widespread, rapid, and intensifying on an alarming scale. Numerous trends, such as sea level rise, are now considered irreversible. The release of this report generated a pronounced reaction from the participants during the Society's Business Meeting. Under New Business, a suggestion was made to develop mechanisms to demonstrate the countless ways members of SIMB are responding to help mitigate climate change. These suggestions will be forwarded to the Society's Board of Directors for consideration.

Wishing the best to all SIMB Members.

Sincerely,

Melanie R. Mormile

Editor-in-Chief, *SIMB News*
mmormile@mst.edu

Letter from SIMB President

Dear Friends and Colleagues,

As we embark on a hopeful path toward post-pandemic normalcy, SIMB is to be commended for their prudent choices in the last year to maintain our resources while remaining in service to our members. A great thanks to my predecessor Steve Decker and the SIMB Board of Directors for making the wrenching but wise decision to cancel the 2020 Annual Meeting. The following SBFC meeting was held virtually, and was successful.

However, people missed attending live meetings, and so with a bit of trepidation, we decided to make the SIMB Annual Meeting live in Austin. We had 270 attendees, which was a good turnout for a year when many other organizations were still meeting virtually. Importantly, the attendees greatly enjoyed it, the talks were of the usual high quality, and it "felt" like a normal meeting. A special kudos to Adam Guss, who started as Annual Meeting chair in 2019, and continued through 2020. He handled all the unpredictable changes with determination and grace.

My goal this year is to expand the benefits we offer our members. Compared to other larger professional societies, one of our hallmarks is that we are very accessible to our members. This is especially important for our younger members to have the opportunity to work side-by-side with senior members at meetings to learn from them. To that end, we aim to:

» Increase the access of our members to each other by setting up social media platforms for interactions. A monthly gathering is proposed on Zoom/Teams to host presentations and discussions. Grad students or others (e.g. SIMB Fellows) can present a talk on a topic or technology. This is a good opportunity to be known among colleagues. Multiple groups can be formed, e.g. one for grad students/young professionals, etc.

» Increase access to the board members, committee chairs, and conference chairs. To offer suggestions on policy, benefits, topics you'd like to see in a meeting. On our website is the contact info (email) for our board members and committee chairs. Write to them to ask questions or offer your suggestions for what SIMB can do for you. This can be in the form of topics for future meetings, how we can help in careers, etc. - *https://www.simbhq.org/about-simb/board-committee-members*

» Expand our career resources for both students/early career members as well as for late career microbiologists (50 to retirement). We are looking to expand our online resources, as well as career workshops at meetings, and chat forums.

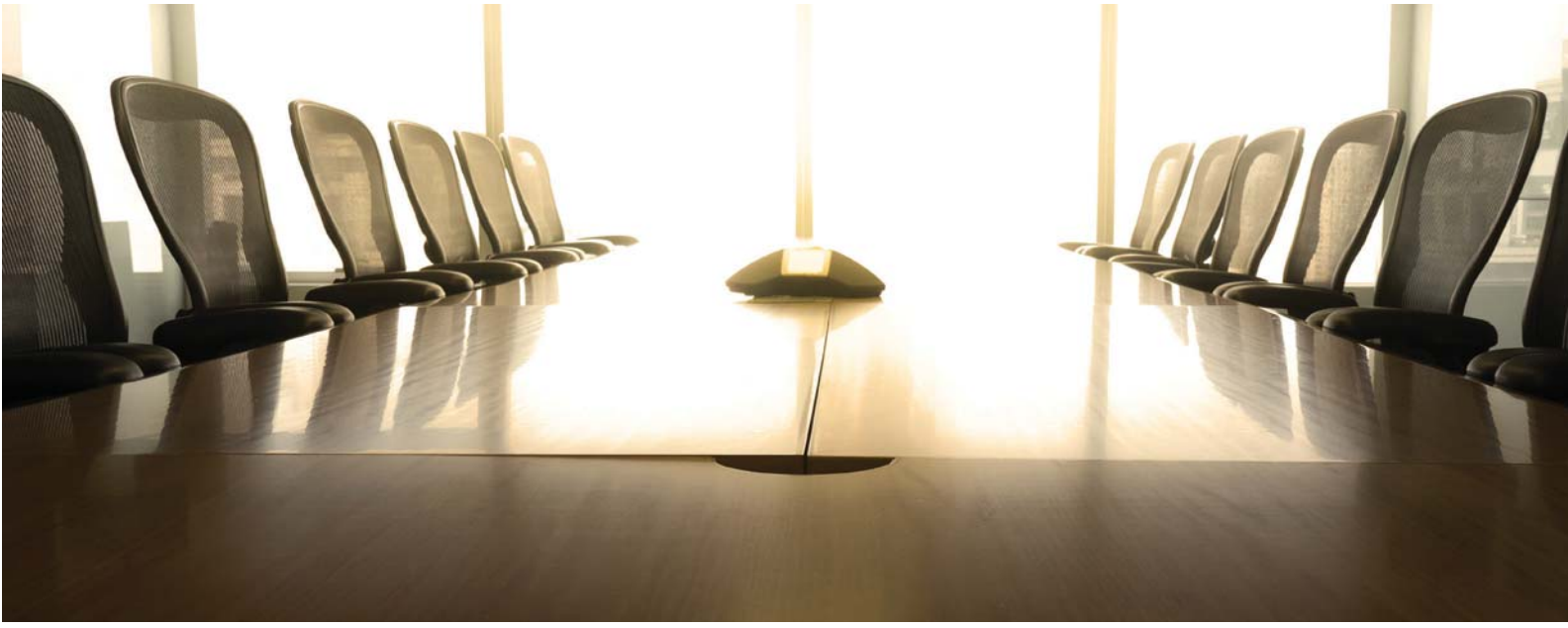
For these efforts, we welcome suggestions and volunteers, so please email us (you can even start by emailing me at *noelmfong@gmail.com*). In the meantime, watch for email announcements and updates in *SIMB News*.

Finally, I know that as I start this year, I am standing on the shoulders of giants. Thanks to the Board of Directors, and a special nod to the outgoing members, Past President Jan Westpheling, and Director Tiffany Rau. As always, a big thanks to Chris Lowe and her hardworking team at SIMB Headquarters.

Best Regards,

Noel Fong
SIMB President
noelmfong@gmail.com

"Biology conferences are groups of cells gathering to talk about other cells."



SIMB Strategic Plan

Vision

To be the leading international professional society in industrial microbiology and biotechnology

Mission

Empower our members and others to address current and future challenges facing humanity using industrial microbiology and biotechnology.

Core values

Scientific excellence (innovation, rigor, multi-disciplinary science and engineering, translational technology)

Leadership (collaboration, continuity, advocacy)

Diversity (promotion, inclusion, openness, internationality)

Responsibility (ethics, integrity, transparency, societal impact)

Communication (education, information, outreach, responsiveness)

Passion for science (fun, inspiration)

Goals

1. Provide information to increase global knowledge, understanding, and application of industrial microbiology and biotechnology.

2. Organize preeminent meetings in our core scientific disciplines.

3. Publish the leading journal in industrial microbiology and biotechnology.

4. Promote and increase diversity in all aspects of the Society, with membership open to anyone interested in our vision and mission.

5. Enhance the value of membership in the Society for both individual and corporate members.

6 Offer educational/professional development opportunities for the membership and the general public.

7. Communicate our activities and accomplishments in industrial microbiology and biotechnology to both the global scientific community and the general public.

8. Expand our global reach.

9. Ensure the financial and operational stability of the Society.

Newsworthy

Bipartisan, Bicameral Legislation Would Support Development of Innovative Antibiotics to Treat Resistant Infections and Improve Appropriate Antibiotic Use

Washington, D.C. – U.S. Senators Michael Bennet (D-Colo.) and Todd Young (R-Ind.) and Representatives Mike Doyle (D-Pa.) and Drew Ferguson (R-Ga.) reintroduced the Pioneering Antimicrobial Subscriptions to End Up surging Resistance (PASTEUR) Act to encourage innovative drug development targeting the most threatening infections, improve the appropriate use of antibiotics, and ensure domestic availability when needed.

"After witnessing the COVID-19 pandemic, it has never been more clear that we need to invest in research to prepare for the next public health crisis," said Bennet. "Infectious disease experts are already sounding alarms, and they need resources to prepare for the threat that antimicrobial resistance infections pose. With our bipartisan PASTEUR Act, we have the chance to not only learn from the mistakes we have made up to this point, but to invest in tools to better prepare for the future."

"Americans understand – now more than ever – that we must take every reasonable and responsible measure to prevent future public health crises. Antimicrobial resistance has become a growing crisis in recent years. Market failures have resulted in a lack of needed research and development in this field which is a threat to public health. That's why I'm proud to reintroduce our Pioneering Antimicrobial Subscriptions to End Upsurging Resistance (PASTEUR) Act to incentivize development of new antibiotics. At the same time, the PASTEUR Act will focus on educating health care providers on how to avoid overuse or misuse of these life-saving medications in order to slow the emergence of antibiotic-resistant pathogens," said Young.

"Tens of thousands of Americans die each year from antimicrobial-resistant infections," said Doyle. "Infectious disease experts agree that antimicrobial resistance is an urgent public health threat that requires a comprehensive, effective solution now. The PASTEUR Act will help scientists and researchers bring better antimicrobials to market, and it will help hospitals and doctors ensure these drugs are used properly."

"Antimicrobial resistance is a looming public health and national security crisis that may one day be our next pandemic if left unaddressed," said Ferguson. "Applying the lessons learned from COVID by investing in pandemic preparedness now will save lives later, which is why I am so proud to be a part of this bipartisan, bicameral effort. We must bring

together the unique capabilities and resources of the public and private sectors to solve the market failures impeding the development of new lines of antibiotics. The PASTEUR Act accomplishes this and I look forward to working with my partners on this legislation towards its enactment."

According to the Centers for Disease Control and Prevention's (CDC) Antibiotic Resistance Threats in the United States report, more than 2.8 million antibiotic-resistant infections occur in the United States each year, and at least 35,000 people die as a result. In March 2015, the U.S. National Action Plan for Combating Antibiotic-Resistant Bacteria directed federal agencies to accelerate a coordinated, full government response to antibiotic resistance and take action to expand the ability of our health care system to prevent, identify, and respond to the infection pandemic threat posed by antimicrobial resistance. Part of this plan was to increase and incent development of innovative antimicrobial drugs to treat resistant infections. Because of severe market failures in the health care system, many of the innovative antibiotic companies doing this work have filed for bankruptcy and stopped producing their critical drugs completely.

The PASTEUR Act would address this market failure and increase public health preparedness by keeping novel antibiotics on the market and improving appropriate use across the health care system. While current contracts between the government and drug makers base payment on volume, the PASTEUR Act would establish a subscription-style model which would offer antibiotic developers an upfront payment in exchange for access to their antibiotics, encouraging innovation and ensuring our health care system is prepared to treat resistant infections.

The PASTEUR Act would:

» Establish a subscription model to encourage innovative antimicrobial drug development aimed at treating drug-resistant infections. This model will be fully delinked, meaning that participating developers would not receive income, as a part of their subscription payments, based on volume or quantity of sales.

» Subscription contracts would contain terms and conditions including product availability to individuals on a government health insurance plan, supporting appropriate use, and completion of postmarketing studies. These contracts could be valuated between $750 million and $3 billion.

- Build on existing frameworks to improve usage of the CDC National Healthcare Safety Network, the Emerging Infections Program, and other programs to collect and report on antibiotic use and resistance data.
- Include transition measures such as smaller subscription contracts to support novel antimicrobial drug developers that need a financial lifeline.

Bennet and Young first introduced the PASTEUR Act in September 2020.

2021 Science Fairs

by ***Thomas Klasson, USDA***

This year, SIMB sponsored two awards at the Virtual 2021 Greater New Orleans Science and Engineering Fair (GNOSEF). GNOSEF was organized by Tulane University in New Orleans on February 22-March 1, 2021, and encourages independent student research in science and engineering; promotes the understanding and appreciation of sciences; encourages youth to pursue science, math, or engineering careers; stimulate interest and support for science and math programs in area schools; promote collaboration and interaction between area students and scientists and engineers from the community and/or the world. The GNOSEF is one of the oldest such fairs in the nation, with the first held in 1956 and hosts about 300 6th-12th grade students annually. SIMB sponsored a 1st place award of a Certificate and a $75 gift card for Junior Division and a $125 gift card for the Senior Division to an individual student who demonstrate the best microbiology or biotechnology related project. This year was a unique experience as there was not an opportunity to speak with the students who were responsible for the projects. Instead, written reports, photos taken during experiments, and sometimes a slide deck was provided for review. Past SIMB Director Dr. K. Thomas Klasson served as judge and reviewed entries by the young scientists who had projects best fitting our criteria. A total of 195 projects were evaluated for their relevance to microbiology and biotechnology.

A student from Lake Forest Charter School in New Orleans, Louisiana, took home the SIMB Award in the Junior Division for the work investigating the difference between the amount of bacteria presence on cell phones as a function of gender. Cell phones from 5 boys and 5 girls were sampled with sterile cotton swabs and steaked onto agar plates and incubated for 10-12 days, after which the number of colonies were counted. The results showed that the cell phones of boys contained more bacteria than the girls' phones. This was contrary to the hypothesis tested, which was based on the assumption that girls use their phones more and use facial products that may support bacterial growth.

A student from Holy Cross School in New Orleans, Louisiana won the SIMB Award in the Senior Division for the work on the quantification of breath bacteria that passes through certain school band instruments. After disinfection of the sound exit section (bell) of a trumpet, baritone, saxophone, trombone, tuba, and French horn, each instrument was played with the same notes for 15 minutes and the bell was sampled with sterile cotton swabs and streaked onto LB medium agar plates. After incubation for three days, the colonies were counted and recorded. The experiment was performed twice and the results showed that there was a clear connection between the length of instrument tubing (1.2-5.5 m) and the amount of bacteria collected on the bell. The longer the instrument tubing, the less bacterial was collected on the bell.

Both of these aspiring researchers were well-deserving of the SIMB Award and have a great future in the field of science. SIMB congratulate them and wishes them all the best.

Erick J. Vandamme

Microbes, Fermentation and Industrial Microbiology: From Antiquity till the early 1900s

1. Impact of microorganisms on planet Earth

Microbes and their activities have been crucial in the deep past for life to emerge and develop on planet Earth and they will remain extremely important for all life on our planet for many millennia and geological eons to come, and long after humans and other life forms have disappeared (Knoll, 2011; Vandamme, 2019, 2022). It is estimated that more than 60% of the Earth's biomass consists of microbes, that 90% of life in the oceans is microbial biomass and that > 5x1031 microbial cells are around. Their activities and overall role in the biogeochemical cycles, mineralization and turnover of biomass are pivotal for all life forms. More photosynthesis is accomplished by photosynthetic bacteria than by green plants and algae. Over 50% of the cells in human bodies are microbial cells (Sender et al., 2016).

Figure 1: Sculpture of Jia Sixie in Weifang Vocational College, China.

Germfree animals are less healthy than those colonized by their microbiomes. All these aspects indicate that life on Earth depends heavily on microbes and on their activities and many of these have been exploited by mankind – for a long time unknowingly – since ancient times! Furthermore, microbes are the system of choice to study evolution since they provide rapid generation times, high genetic flexibility, unequaled experimental scale potential and they are also a source of a wide range of valuable metabolites and enzymes (Vandamme, 2016, 2022; Demain et al., 2017). Study and exploitation of microbes to produce such useful compounds to the benefit of humankind has been referred to over time as industrial fermentation, industrial microbiology and microbial biotechnology (Baltz et al., 2010; Soetaert and Vandamme, 2010; Vandamme and Revuelta, 2016; Baltz et al., 2017).

2. The hidden beginnings of fermentation and industrial microbiology

The practice of fermentation, industrial microbiology and biotechnology has its roots deep in antiquity. Especially production and preservation of fermented foods and alcoholic drinks were practiced for centuries and became a respected craft in society. These "spontaneous" processes were gradually improved by trial and error - and often seen as an art - and contributed unknowingly to the nutrition and health of people. Indeed, long before their discovery, microorganisms were unknowingly exploited to serve the needs and desires of humans, i.e., to conserve fruits, vegetables, grains and milk, and to enhance the quality of life with the resultant fermented drinks and foods, i.e., wine, beer, fermented plant and fruit juices, mead, yoghurt, kefir, cheeses, bread, vinegar, soy sauce (shoyu), kimchi and many other "pickled" foods (Demain and Solomon,1981; Prajapati and Nair, 2003; Smith, 2020).

Also, nonedible crude biomaterials such as plant fibers and animal hides were "fermented" to arrive at stronger binding and packing materials (retting of flax, hemp, coconut, etc.) or better clothing and shelter (tanning of animal skins). The oldest fermentation know-how, the conversion of sugar to alcohol by yeasts, was used to make beer in Sumer and Babylonia even before 7000 BC (Hardwick, 1995). The importance of fermented foods and drinks is reflected in the fact that the Sumerians offered since 3500 BC beer to Goddess Nin-Kasi, that Egyptians stored beer in their tombs and that the Greco-Roman mythic deity, named Dionysos or Bacchus, was seen as god of fertility, good grape harvest and wine making. Hammurabi's code (1754 BC) mentions laws related to daily beer use by citizens and beer merchants. By 4000 BC the Egyptians had discovered that carbon dioxide - generated by the action of brewer's yeast as we now know - could leaven bread. By 100 BC, ancient Rome had over 250 bakeries which were making leavened bread. Wine was made in China as early as 7000 BC and in Assyria since 3500 BC. Ancient people made cheese thereby unknowingly preserving milk with molds and bacteria as a method of preservation. Milk was fermented with lactic bacteria to form lactic acid as a in situ preservative to make yoghurt. Milk was also converted into kefir and kumiss using *Kluyveromyces* yeast species in Asia. In the Far East, the use of molds to saccharify rice in the "koji" process dates back at least to 700 BC. Rice vinegar, soy sauce, soybean paste (miso), rice wine (sake), natto and tempeh are as old and are fermented foods still very common in Japan, China, South Korea and other East Asian countries. In China famous scholar and agronomist Sixie Jia (Figure 1) wrote in the period 533-544 AD (during the Northern Wei Dynasty) a monograph, entitled "Qimin Yaoshu" (Essential Skills for the Welfare of People) on essential agricultural and fermentation practices, mentioning also over 20 methods to brew rice vinegar. Vinegar and many other fermentation products were and still are very important in China and in the Far East since a very long time (Smith, 2020). In Europe vinegar manufacturing from wine began in Orleans, France, only towards the end of the 14th century AD and spread all over. Cider, mead and beer were also fermented into vinegar (Demain and Solomon,1981; Smith, 2020). By that time the distillation of alcoholic spirits from yeast fermented grain, a practice thought to have originated in 900 AD in China or the Middle East, was common in many

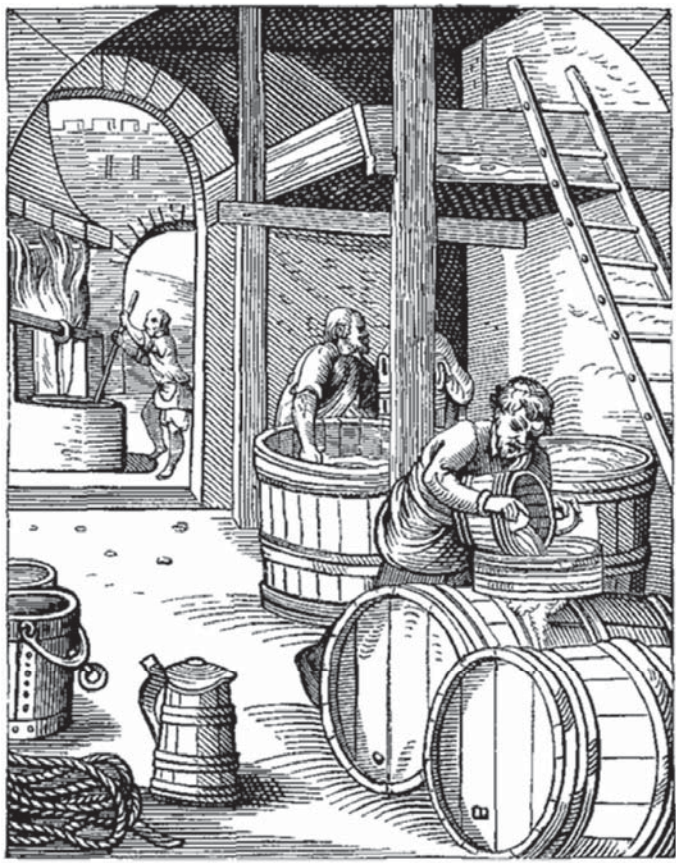


Figure 2: An engraved representation of a brewery in the 16th century.

parts of the world. It had started in Europe in the late 11th century in Salerno, Italy. In Europe over time these fermentation processes had an ever-increasing economic impact, with products such as cheeses from milk, wine from grapes in warmer Mediterranean societies, while beer from grain was important in what is now Northern and Western Europe. These ancient fermentations went on throughout the Middle Ages (500-1400 AD) and the Renaissance (1400-1600 AD), with abbeys and cloisters in Europe as being the centers of knowledge where the processes were improved and included the use of manuscripts and records to describe these processes. Many famous woodcuts, paintings and drawings depicted stages of malting and brewing. It became an esteemed profession that gradually grew into an economic force in society.

Today's beers brewed from malted grains and hops have little in common with the drink with that name common throughout the European Middle Ages and the

Figure 3: Anthonie van Leeuwenhoek

Renaissance. See Figure 2 for an engraved representation of a brewery operating in the 16th century. Then it served often as a safe and cheap nutritional necessity, as compared to consuming contaminated water or expensive wine. It was often flavored with all kinds of herbs, and consumed by men, women and children alike. Beer became a commodity drink of economic, health-promoting as well as social importance. Furthermore, it was a major source of tax revenue as was vinegar for the government authorities and both were also in use as a medicine (Unger, 2004; Smith, 2020). The Assyrians treated chronic middle ear diseases with vinegar, and Hippocrates of Kos (460-377 BC) treated patients with it in 400 BC. For thousands of years, moldy cheese, meat and bread and warmed soil were employed in folk medicine to heal wounds. It would take over 2 millennia to find out the base of this healing effect with Alexander Fleming's discovery in 1928 of penicillin produced by the fungus *Penicillium notatum* (Brown, 2004). It took until the findings of the 17th to 19th century to realize that specific microbes were essential to arrive at controlled fermentations for a range of fermented foods and drinks, and for a wide spectrum of useful bulk and specialty biochemicals, enzymes and antimicrobial agents.

The major progress made from 1700 onwards till the first decades of 1900 is detailed here. Several historical, political, sociological and scientific events as well as personal rivalry among famous scientists played a crucial role in this very productive period of applied microbiology and fermentation. Examples are battles and wars, epidemics, raging animal and human diseases, widely spread food spoilage and drinking water contamination, failing traditional brews, spoiled beer, layman wisdom versus elite-scientists' disdain, conflicting opinions, wild theories, development of novel laboratory and industrial techniques and also foresight as well as sheer luck. Some of these events and rivalries are interwoven in the text below whenever relevant.

The enormous strides taken by fermentation, industrial microbiology and industrial biotechnology, since the early 1900s up till the early 1940s, have been summarized recently by the author (Vandamme, 2016). More recent developments since the 1950s can be found in comprehensive reviews and books (Soetaert and Vandamme, 2009; 2010; Baltz et al., 2010; 2017; Demain et al., 2017; Vandamme, 2019; Vandamme and Mortelmans, 2019; 2020).

3. Increasing impact of science and technology on microbiology and biochemistry (1673-1920s)

3.1. Gradual disbelief in spontaneous generation (period 1673-1860)

In the 17th century, the Dutch drape-merchant with no university training Anthony van Leeuwenhoek (1632-1723) (Figure 3), living in Delft, The Netherlands, had as spare-time interest in the construction of simple lens microscopes. He reported in 1673 on the presence of tiny "animalcules", i.e., moving small organisms "less than a thousandth the size of a grain of sand" by using his simple lens to the examination of water, decaying matter, blood and scrapings from his teeth. In 1676 he observed "incredibly small" organisms, most probably bacteria, and in 1680 small spherical globules (among them yeast cells), but did not consider these as living cells. The lack of university connection might have caused his discoveries to go unknown. The secretary of The Royal Society in England, Henry Oldenburg who corresponded with

European science amateurs, among them Leeuwenhoek's friend, the Dutch physician Regnier de Graaf (1641-1673) made the connection! As a result, from 1673 to 1723, van Leeuwenhoek's great powers as a microscopist were communicated to the Society in a series of letters.

In the preceding centuries, most scientists thought that small organisms such as worms, insects and snails arose spontaneously from nonliving matter (Vandamme, 2019). This theory of spontaneous generation was originally postulated by the Greek natural philosophers Anaximander of Miletus (610-540 BC) and by Aristotle (384-322 BC) and advocated heavily by the Greek physician Galen (129-ca. 200 AD), who also had proposed the "miasma" theory, stating that disease transmission was caused by "bad air" or vapor emanating from rotting and decaying organic matter. What followed was a 200-year argument over spontaneous generation, also called the "War of the Infusions" (Vandamme, 1992; 2019). Proponents had previously claimed that maggots were spontaneously created from decaying meat, but this was discredited first by Italian natural philosopher and biologist Francesco Redi in 1668, showing that maggots come from fly eggs (Hawgood, 2003), and 100 years later by Italian physiologist Lazzaro Spallanzani in 1768 in preventing clouding (in hindsight by growth of microbes) of nutrient beef broths by boiling them in sealed containers (Nordenskiöld, 1935; Vandamme, 2019). By this time, the theory of spontaneous generation was discredited with respect to higher forms of life, so the proponents concentrated their arguments on microbes (Strick, 1997). The theory did seem to explain how a clear nutrient broth became cloudy via growth of large numbers of such "spontaneously generated microorganisms" as the broth aged. However, others believed that microorganisms only came from previously existing microbes and that their ubiquitous presence in air and on fomites was the reason that they would develop in organic infusions after gaining access to these nutrient-rich liquids. Three independent investigators, French physicist Charles Cagniard de la Tour (1777-1859), German physiologist Theodore Schwann (1810-1882) and German botanist Friedrich T. Kützing (1807-1893) proposed in 1837 that the products of fermentation, chiefly ethanol and carbon dioxide, were created by yeast cells, a microscopic form of life (Aszmann, 2000; Vandamme, 2019). This concept was bitterly opposed by the leading chemists of the period such as Swedish chemist Jöns Jakob Berzelius (1779-1848) and German organic chemist Justus von Liebig (1803-1873), who believed in "vitalism" and considered fermentation to be strictly a chemical reaction. They maintained that the yeast in the fermentation broth was lifeless decaying matter. Organic chemistry was flourishing at the time, and these opponents of the living microbial origin of fermentation were initially quite successful in putting forth their views (Jorpes, 1970; Brock, 1997).

3.2. Disproof of spontaneous generation by Pasteur and Tyndall and birth of microbiology and biochemistry as new disciplines (1860-1897)

Decisive experiments were soon to settle forever since the 25 centuries lingering dispute about spontaneous generation and miasma. In 1860-1861 French chemist-microbiologist Louis Pasteur (1822-1895) proved the omnipresence of microbes in air with his simple but famous "swan neck open flask" series of experiments that discredited the theory of spontaneous generation of microbes all together or that "spontaneous fermentation" was caused by microbes. Until that time he was challenged and opposed by the views of the renowned French naturalist Felix A. Pouchet (1800-1872), who firmly stated that living things and germs originate from inanimate matter, air, unrelated or once living organisms, his concept being called "heterogenesis" (Farley and Geison, 1974). Pasteur's findings were further supported by experiments about 15 years later by Irish physicist John Tyndall (1820-1883). He was a correspondent and admirer of Pasteur, who contributed to the complete fall of spontaneous generation in the period 1876-1881 by developing a method for fractional sterilization named "Tyndallization" of broths that also killed bacterial endospores. These normally survive boiling as demonstrated in 1876 by botanist Ferdinand Cohn (1828-1898). In hindsight Pasteur must have had sheer luck with his swan neck open flask experiments in that no endospore forming bacteria spoiled his tests. Tyndall described his experiments in a book "*Assays on the Floating Matter of the Air in Relation to Putrefaction and Infection*" "(Tyndall, 1881; Eve and Creasy,1945). Both scientists demolished the concept of spontaneous generation and proved that existing microbial life came from preexisting microbial life. It was at this point that

Figure 4: Moritz Traube

microbiology was born, but it had taken about two decades to disprove the chemical hypothesis of Berzelius and colleagues i.e., that fermentation was the result of contact with "decaying matter".

In 1848, Pasteur then at the Faculty of Sciences in Strasbourg, France, had studied the stereochemistry of crystals and was able to demonstrate the dextro-and levo-form of tartaric acid crystals in wine. This led him in 1854 to become Professor and Dean of the Faculty of Sciences in Lille, a city in Northern France, and to focus on the study of living microorganisms (bacteria and yeast), that enabled him to carry out different fermentations, many of them being of economic importance for the wine and beer industries in France. Interest in the mechanisms of these fermentations resulted in later investigations by Pasteur and contemporaries, that not only advanced microbiology as a distinct discipline, but also led to the development of vaccines and concepts of hygiene which revolutionized the practice of fermentation, medicine and sanitation (Vandamme, 2019; Vandamme and Mortelmans, 2020).

In 1877 German chemist and physiologist Moritz Traube (1826-1894) (Figure 4) proposed that enzymes are protein-like materials, catalyzed fermentation and other chemical reactions, and that they were not destroyed during such activities. This was the beginning of the concept of what is called enzymology and biochemistry today. He also proposed that fermentation was carried out via multistage reactions in which the transfer of oxygen occurred from one part of a sugar molecule to another, finally forming some oxidized compound like carbon dioxide and a reduced compound such as alcohol (Sourkes,1955). The field of biochemistry became established in 1897 when German chemist Eduard Buchner (1860-1917) found that cell-free yeast extracts, lacking whole cells, could convert sucrose into ethanol. Thus, the views of Pasteur were modified and it became understood that "fermentation" could also be carried out in the absence of living cells, but by using their enzymes as biocatalysts.

3.3. Microbiology meets with technology: birth of enzymology and biocatalysis industrial microbiology, medical microbiology, vaccination and immunology, and antibiosis (1833-1928)

3.3.1. Pioneer scientist at the origin of enzymology and biocatalysis (1833-1926)

Several practical enzyme-based developments date from middle 19th century. Examples are the use of diastase (amylase), extracted from malted barley in the brewing industry (Payen and Persoz, 1833) and of calf's rennet, Emil Christian Hansen's preparation for cheese making (1874). The Japanese scientist Jokichi Takamine (1854-1922) (Figure 5), while working in the USA, was the first to patent in 1894 a microbial enzyme product. His "Takamine" process involved extraction of extracellular amylases with aqueous ethanol from the mold *Aspergillus oryzae* grown on bran, a process similar to the ancient Japanese koji-process (Takamine, 1894). He modified the traditional solid state culture process for industrial production of a mixed enzyme preparation named "Taka-Diastase" containing amylases and other extracellular enzymes. He applied the preparation first

to the production of alcoholic beverages from grains and then for the treatment of dyspepsia or indigestion. This was a pioneering effort towards the application of microbial enzymes. It also heralded the trend to replace enzyme resources from higher plants or animals for microorganisms.

More technical developments based on enzymes started at the onset of the 20th century with the foundation of the Rohm & Haas company in 1907 in Germany when several practical enzymatic reactions were described, based on crude preparations of amylase, lipase, protease, trypsin, pepsin, and invertase. For leather manufacturing early tanners kept the animal skins in a warm suspension of dog and bird dung, not knowing that the unpleasant bating practice was based on the action of enzymes (pepsin, trypsin, lipase) present in the animal dung. Once this mechanism was revealed in 1898, soon a bacterial bate was developed from *Bacillus erodiens* cultures and commercialized as a bacterial culture "Erodin" adsorbed on wood meal, the first immobilized biocatalyst. In 1907, pancreatic extract was introduced as a bating agent by pharmacist Otto Rohm (1876-1939) who founded with Otto Haas his own company, Rohm & Haas, in 1909 in Esslinger, Germany. With the trade name "Oropon" his product became very successful and he moved production to larger facilities in Darmstadt, Germany. It was a place with a growing market searching for a new and pleasant technical product. That was an important factor in his success. Also in the Netherlands, the company Organon launched a similar product, named "Leeropaan."

Early in the 20th century plant lipases were produced by mechanical disruption of *Ricinus communis* seeds (castor beans) and were used to produce fatty acids from oils and fats. It was also found that this reaction is reversible and the enzymatic synthesis of fat from glycerol and fatty acids had been described as early as 1911. Proteolytic enzymes had been successfully used in the USA since 1911 for the chill-proofing of beer. Wheat diastase was found to interact beneficially with dough making and the addition of malt extract became a common practice in bread baking. A few years later, a soaking agent "Burnus" containing enzymes that facilitated laundering was introduced, followed in 1920 with enzyme-based wound care products. Enzymes were then also introduced in the textile and silk industry and in the manufacturing of hide glue. In 1934 enzymes gradually infiltrated the food industry for clarification of apple juice and later on in baked goods. Production of pectinases started in Europe around the 1930s for use in the fruit juice sector.

The scientific background on the functioning and use of enzymes, to be seen as catalytic proteins, only emerged in the late 19th century. This knowledge was based on German chemist Emil Fischer's (1852-1919) findings in 1894 on enzyme specificity and its "lock and key" action (Fisher 1894, 1909) and on the joint experiments of the brothers Eduard (as a chemist) (Figure 6) and Hans (as a bacteriologist) Buchner (Buchner, 1897) on the pure chemical nature of the alcohol fermentation in the absence of living yeast cells. Eduard Buchner, Chemistry Nobel Prize winner in 1907, called the soluble agent in his yeast press juice "zymase". Their work killed the "vis vitalis" (vital force) paradigm altogether. Kinetic studies, published in 1913 by German chemist Leonor Michaelis (1875-1949) at Berlin University and his Canadian biomedical student Maud Menten (1879-1960), were also very important towards the understanding

Figure 5: Jokichi Takamine

Figure 6: Eduard Buchner

of the physicochemical nature and kinetics of enzyme action (Michaelis and Menten, 1913). A further key step towards the "chemical paradigm" was the work of James B. Sumner in 1926 on the crystallization of jack bean (*Canavalia ensiformis*) urease and on the protein nature of enzymes. In the 1930s, several more enzymes were isolated, purified, and crystallized from plants, animal organs, yeast, fungi and bacteria (Sumner and Myrback, 1951). The development in the 1920s of large-scale submerged fermentation processes for citric acid and for penicillin in the early 1940s did not immediately lead to increased industrial fermentation production and applications of microbial enzymes. These changes were not realized until the late 1950s with the emergence of detergent enzymes, the industrial use of glucoamylase to produce glucose from starch and the potential of (hemi) cellulases to deconstruct biomass into fermentable sugars (Demain et al., 2005; Vandamme, 2016; Baltz et al. 2017).

3.3.2. Pioneer scientists at the origin of controlled industrial fermentation processes for beer brewing, wine making, alcohol and (bio)chemicals (1856-1898)

In 1856 beet sugar-distillers of Lille, France, called upon Louis Pasteur, then Chemistry Professor at the University of Lille, to find out why the contents of their alcohol fermentation vats were turning sour. He noted through his microscope that the fermentation broths contained not only yeast cells, but also bacteria, initially called "lactic yeast", that could convert the beet sugar into lactic acid, lowering the alcohol yield drastically. This phenomenon was causing an economic loss to the region. He introduced careful microscopic examination of the microbial cultures in use and applied heat treatments to minimize this "disease". Inspired by these practical observations, Pasteur focused on other fermentations and he established that each type of fermentation (alcoholic, lactic, and butyric) was mediated by a specific microorganism. His "lactic yeast" responsible for the formation of lactic acid was noticed in 1883 by Charles E. Avery, an MIT educated chemist, who developed the first lactic acid fermentation plant, the Avery Lactate Company, in Littleton, MA, USA, using hydrolyzed corn starch as fermentation substrate. Lactic acid was to be used as an acidulant in the bakery sector to replace the chemical potassium bitartrate. A fire ruined this successful plant in 1911 and in the early 1900s several competitors in the USA, Europe and the UK took over with whey, molasses or sugar as a substrate and use of pure *Lactobacillus* sp. cultures (Whittier and Rogers, 1931; Benninga, 1990). Under World War 1 (WW1) pressure, German production capacity was increased to meet the military demand. Later technical uses, other than food grade uses, came into practice worldwide. These D- and L-lactic acid fermentation processes are thriving even today for food, chemical and biodegradable plastic applications!

In 1857, Pasteur returned to his Alma Mater "l'Ecole Normale Superieure de Paris", a school of highest educational level in basic sciences and engineering. Already in 1861 he reported butanol and acetone being formed as fermentation products, later to be shown being produced at high levels by *Bacillus* and *Clostridium* bacteria (Schardinger, 1905; Weizmann, 1919). This finding would be exploited by Chaim Weizmann in the United Kingdom under WW1 pressure to develop a commercial fermentation process on cereal feedstock for acetone, butanol and ethanol (ABE) formation, much needed chemicals for ammunition and as solvents (Vandamme, 2016).

Furthermore, in a study undertaken to determine why French beer was inferior to German beer, Pasteur demonstrated in 1863 the existence of strictly anaerobic life, i.e., bacterial life in the strict absence of air. In 1864 he was able to prevent souring of wine and beer upon ageing by applying a mild heat treatment technology,

which later became known as "pasteurization", and is still universally used in the dairy and food processing industry. In 1867, he observed that "*Mycoderma aceti*" bacteria (mother of vinegar) oxidized the alcohol in wine into acetic acid to convert it into vinegar. United Kingdom's brewing chemist Adrian J. Brown (1852-1919) used later similar acetic acid bacteria "*Bacterium aceti*" (now *Acetobacter aceti* subsp. *xylinum*) to oxidize alcohols such as mannitol to fructose, propanol to propionic acid, and also proved that these bacteria formed a mat of cellulose fibers (Brown, 1886). French biochemist Gabriel Bertrand (1867-1962) also reported on oxidation of polyols to ketones using Brown's strain (Bertrand, 1898). These observations would later in 1934 lead to a commercial bioconversion process of D-sorbitol to L-sorbose, an essential step in the chemoenzymatic route to large scale vitamin C production (Reichstein and Grussner, 1943; Wells et al., 1937; Vandamme and Revuelta, 2016) and to the production of pure bacterial cellulose via fermentation of sugars for food, medical and technical applications (De Wulf et al., 1996).

In 1879, a coworker of Pasteur, Charles Chamberland (1851-1908) (Figure 7), developed unglazed porcelain filters, with pores smaller than the size of bacteria, though not of that of viruses, allowing to "sterilize" liquids without heating and to isolate and propagate viruses. His technology skills also led in 1884 to the development of the autoclave, now universally in use and essential in research and in industries, related to the microbiology, biotech, fermentation, pharma, medical, food and sanitation sectors.

In the late 19th century, inspired by Pasteur's many crucial achievements, several renowned scientists believed that the emerging industrial application of microbiology would form a new type of industry distinct from the then rapidly growing petrochemical industry. This idea was, at least in Europe, based on the huge importance and value of the German beer industry at the turn of the 19th century. Brewing was second only to machinery building and surpassed metallurgy and coal mining. Indeed, based on Louis Pasteur's theories and practical findings in France, combined with the efforts of Robert Koch and Ferdinand Cohn in Germany, and the work of Emil Christian Hansen in Denmark, beer brewing had evolved from an art into a controlled and well understood malting, mashing, and yeast fermentation process. At that time yeast culture collections were established in Prague, Delft, and Berlin with fermentation and brewing research institutes founded, in Paris, Copenhagen, Vienna and Berlin (Pasteur Institute, Paris; Carlsberg Institute, Copenhagen; Vienna Technical Institute, Vienna; Institut fur Gärungsgewerbe (Institute for Fermentation Industries), Berlin. These institutes soon gained impact and fame that last until today, be it with other names! In 1898, an English translation of Franz Lafar's (1865-1938) famous two-volume German handbook "Technical Mycology: The Utilization of Microorganisms in the Arts and Manufactures" (Lafar, 1898) became widely available. Lafar, the first director of the Vienna Technical Institute, Vienna, Austria, became renowned as a result of his improvements on alcohol fermentation and distillery practice. This would eventually also lead to the large-scale fermentation of ethanol as a biofuel for transportation and as a disinfectant up till today.

Figure 7: Charles Chamberland

Figure 8: Robert Koch

3.3.3. Pioneer scientists at the origin of medical microbiology, vaccination and immunology (1798 – early 1900s)

With the establishment of the germ theory of (infectious) disease in 1876 by German physician and bacteriologist Robert Koch (1843-1910), (Figure 8) the last decades of the 19th century were also characterized by the fight against disease and the attention of microbiologists was directed to the medical and sanitation aspects of microbiology. Robert Koch graduated as a medical doctor in 1866 at the University of Göttingen in Germany. In 1871 young Robert Koch, then working as a district medical officer at his primitive home-laboratory in Wollstein, Polish Prussia, not only treated his patients, but devoted also interest to the diseases that killed their farm animals, anthrax being a prevalent killer. His ensuing first ever detailed microscopic and photographic studies of bacteria, his staining procedures for bacteria and use of sliced potatoes (and subsequently gelatin and finally agar on a flat glass plate under a bell jar) as solid culture medium allowed him in 1876 to isolate a spore forming *Bacillus* strain, shown to be the cause of anthrax. Koch used this new solid medium technique to isolate the tubercle bacillus, *Mycobacterium tuberculosis* (Koch, 1882). The petri dish was developed and introduced in 1887 in his lab by his co-worker Julius Richard Petri (1852-1921) (Figure 9) (Petri, 1887; Vandamme, 2018a). In the 1870s Professor Ferdinand J. Cohn (1828-1883), who had already earned a solid reputation as a botanist at Breslau University (now Wroclaw in Poland), also discovered the existence of heat resistant bacterial endospores in 1877 (Vandamme, 2013). This led modest Robert Koch to ask him his opinion on his own studies and results on the etiology of the anthrax bacillus. Koch visited him in Breslau and demonstrated there his innovative experimental microbiological techniques. Cohn immediately recognized the high quality and importance of Koch's work that later influenced his career. Koch's article on *Bacillus anthracis* was subsequently published in Cohn's botany journal "Beitrage zur Biologie der Planzen" in 1876. These new techniques and experiments allowed him to formulate in 1876 a set of rigorous criteria, known as "Koch's postulates" a logical proof of the germ theory of disease. He eventually discovered the etiological agents of over 20 infectious diseases, including anthrax, tuberculosis (Koch, 1882) and cholera with Vibrio cholerae as the causative agent in 1884. Koch, by then a world authority on infectious diseases, was appointed in 1880 at the age of 37 Director of the new laboratory of Bacteriology at the Imperial Health Office in Berlin, Germany, to become in 1885 Director of the Institute of Hygiene at the University of Berlin. At both locations, he recruited a staff of famous scientists, some of them becoming Nobel laureates. Koch was opposed by a renown Bavarian chemist hygienist, Max von Pettenkofer (1818-1901), Director of the Institute of Hygiene in Munich, Germany. He was rightly so an influential proponent of good hygiene, of clean water provisions in cities, and of proper waste disposal, but he did not accept the novel Koch concept that bacteria are a main cause of putrefaction and diseases. In the end, he had to concede and took his own life (Locher, 2007). Specializing with Koch in Berlin in 1883 was the Belgian medical bacteriologist Emile Van Ermengem (1851-1932). He became famous for his discovery and studies in 1895-1896 on the causative agent of botulism. He isolated the anaerobic spore former *Clostridium botulinum* and the botulin toxin (botox) and later developed vaccines in his

Figure 9: Julius Richard Petri

lab at Ghent University, findings still of high relevance to the medical sector and food industry (Van Ermengem, 1897). Botox, neurotoxin A, is now increasingly used in the medical and cosmetic sector to treat muscle contraction disorders (Johnson, 1999; Vandamme, 2012). In 1891, Koch became Director of the Prussian Institute for Infectious Diseases that after his death was renamed as the Robert Koch Institute. During this period, he became interested in tropical diseases of man and animals and undertook several scientific missions in Africa and India. In the 1880s, Koch and Pasteur, both giants in their field of microbiology, became involved in mutual personal rivalry attacks, especially related to isolation and attenuation/ vaccination techniques of the anthrax bacterium *Bacillus anthracis* (Ullman, 2007; Goddeeris,2018). In 1905, Koch received the Nobel Prize in Physiology or Medicine.

From 1867 onwards, Pasteur, now at his new lab of Physiological Chemistry at "l'Ecole Normale Superieure de Paris", took up the study of the origin and spreading of the mysterious silkworm disease ("pepper disease" and "flacherie", caused respectively by parasitic protozoal and microbial agents), that devastated the silkworm nurseries in France, spreading further into Europe and reaching China and Japan. He was able to present methods for curing these insect diseases and this might have led him to deal later on with animal and human disease. However, another French colleague Antoine Béchamp (1816-1908), Professor of Medicinal Chemistry and Pharmaceutics, at the Medical Faculty of Montpellier, became a rival to Pasteur as he had suggested similar curing methods earlier in 1865 by selecting visually non-contaminated silk moths or by creosote treatment. In this frictional situation Pasteur (Figure 10) focused from 1875 onwards rather on medical than on agricultural or (attenuated) pathogenic strains and between 1880 and 1886 on making the individual immune to the disease by vaccination, for instance by injecting either dead or attenuated forms of the disease producing microbes. Examples are fowl cholera caused by *Pasteurella pestis*, anthrax caused by *Bacillus anthracis*, erysipelas caused mainly by *Streptococcus pyogenes* and rabies caused by the rabies virus. In developing and applying these vaccines he was criticized by his medical colleagues including Koch since Pasteur, a chemist and microbiologist, was not trained as a medical doctor. His efforts in developing these safe vaccines as well as the fierce competition with his contemporaries, including Koch and Toussaint, have been described in great detail elsewhere (Dubos, 1960; Geison, 1995; Ullmann, 2007). In 1888 the Pasteur Institute was opened in Paris to honor all his scientific realizations and his broad impact on society. From then on development and application of vaccines became a specialty discipline. In 1886 in the USA Theobald Smith (1859-1934) and Daniel E. Salmon (1850-1914) reported on a heat killed cholera vaccine that protected pigeons. Several killed whole cell vaccines, typhoid fever, cholera, and plague, were soon tested by British troops in India and in South Africa during the Boer War. By the turn of the century human attenuated or killed virus and bacterial vaccines such as vaccinia virus, rabies, and typhoid fever, became widely available and they were massively used during WW1. Pathogenic bacteria and derived toxins and vaccines were produced in "controlled" fermentation vats and then further processed. Toxoids and the BCG vaccine (*Mycobacterium bovis*, attenuated bovine Bacillus Calmette-Guerin) were developed in the early 1920s, use of embryonated chicken eggs in 1931 and animal and human cell line cultures for virus particle production realized in 1949. Production of bacterial and recombinant yeast derived vaccines relied on earlier available bioreactor and fermentation technology, with contained fermentation processes becoming essential. In hindsight the development, large scale production and safe administration of vaccines to very large groups of individuals, occupied much of the early and late research in medical and in industrial microbiology.

It should be mentioned here that Pasteur and Koch and their contemporaries could rely on earlier attempts of vaccination procedures, practiced and recorded since the early 1700s. A practice of folk medicine called "inoculation" (meaning to graft, cutting the skin and rubbing dried smallpox-scab material from a victim in the wound) or "variolation" derived from the name for smallpox "variola", from Latin "varus" meaning pimple was very common. It was however very risky since smallpox, a viral disease as we know now was disfiguring populations with no respect as to social class or age ever since humans domesticated animals (Sherman, 2007; Vandamme and

Figure 10. Louis Pasteur

Mortelmans, 2020). Variants of this technique were used in China, India, the Middle East, and Africa for a long time (Cruse and Lewis, 2005). Variolation came to Europe via a Nottinghamshire duke's daughter Lady Mary Pierrepont. She contracted smallpox in 1715 but recovered, with her face marred forever. When her husband diplomat Edward Wortley-Montagu was to become British ambassador to Turkey she accompanied him and learned there the Turkish practice of variolation. Upon returning to England in 1718, she propagated the technique strongly and, after initial experiments on condemned criminals and orphaned children, could even convince the Royal family to take variolation as a precaution. However, she met with opposition by the clergy and by local physicians. In the English colonies in America variolation was introduced successfully during a Boston smallpox outbreak in 1721 by physician Zabdiel Boylston. This was urged by clergyman and scholar Cotton Mather, a Fellow of the Royal Society of London, who had learned about the technique from one of his African slaves. Again, his fellow physicians were opposed, despite half of the population being infected with a 15% mortality rate.

A first real vaccine was developed by the English country physician Edward Jenner (1749-1823) (Figure 11). He was inspired by a local folktale and turned it into a reliable vaccination technique. Farmers in his Gloucestershire practice in the UK had observed that dairymaids, that had contracted cowpox that was mild in humans, but causing severe blisters on the skin and udders of cows, were resistant to smallpox and did not develop the disfiguring pock-marked faces of smallpox victims. Dorset farmer and cattle breeder Benjamin Jesty contracted in 1774 cowpox from his herd, unknowingly immunizing himself against smallpox. He deliberately inoculated his wife and children with cowpox via scratches and adding cow's udder pox lesions in their arms. They remained immune when later exposed to smallpox. Jenner had heard about the farmer's tale and carried out similar experiments in a systematic and controlled way over a 25-year period. In 1796 he took fluid from a pustule on the wrist of milkmaid Sarah Nelms who had, an active case of cowpox, and smeared it onto the skin of 8-year-old James Phipps, his gardener's son. Six weeks later to test the ability of his "vaccine" (from the Latin name "vacca" for cow) he deliberately inoculated the boy with material from a smallpox pustule, and only a very mild infection reaction and no disease at all resulted. James was "immune" to smallpox! Jenner repeated this treatment (vaccination) about 20 times over the next years and the boy never contracted the disease. He used the hide of cow "Blossom", as a cowpox-source for most of his experiments; it is kept in a glass case in the library in St. Georges Hospital in London, up till today! Jenner wrote down his experiments, but his manuscript was rejected by the Royal Society since he was a simple country doctor and not known by the scientific establishment. In 1798 he published a 70 pages pamphlet, entitled *"An Inquiry into the Causes and Effects of Variolae Vaccinae (smallpox of the cow)"*, stating that inoculation with cowpox produces a "mild form of smallpox", that protects against severe smallpox, as did the risky variolation. He observed that the "disease produced by vaccination would be so mild that the infected individual would not be a source of infection to others", a statement of immense importance. Again, reactions to Jenner's pamphlet were to come in slowly and most physicians rejected his ideas. At the turn of the century, the advantages of vaccination over variolation became clear and the "Jennerian technique" became accepted and used by many physicians. From 1802 onwards he received several financial British government awards and received honors worldwide. It became difficult to obtain sufficient cowpox lymph or to ensure its potency during shipping over long distances. To ensure this need the British Animal Vaccination Establishment was erected, where calves were deliberately infected with cowpox to then collect the lymph as vaccine, later to be stabilized and preserved by adding glycerol. This "glycerinated calf's lymph" was widely distributed from 1895 onwards (Baxy, 1999). The principles behind this acquired immunity were soon to be discovered in the late 1890s.

Figure 11. Edward Jenner

Based on the foundations of Edward Jenner's, Louis Pasteur's and Robert Koch's achievements, it was discovered in the 1890s by pupils of Koch, two Germans, Emil Adolf von Behring (1854-1917), Nobel Prize laureate in 1901, and Paul Ehrlich (1854-1915) (Figure 12), Nobel Prize laureate in 1908, and by the Japanese Kitasato Shibasaburo (1856-1931) and by Russian zoologist Elie Metchnikoff (1845-1916), Nobel Prize winner in 1908 (who had moved in 1888 to Pasteur's lab), that the body's own defense mechanisms, including phagocytosis, played an important role in fighting pathogenic microbes. They found that upon invasion of a human or an animal by a bacterium or virus, proteins (i.e., antibodies) were formed in the blood stream which could specifically neutralize the invading "parasite". All this research led to the foundation of the science of immunology and to improved vaccines (Cruse and Lewis, 2005). In hindsight, folk tales, rural experiences, countryside practice and traditions, stories spread by returned foreign travelers, ("citizen" science avant la lettre) had provided a practical "solution" and were even able to counter the initial opposition and distrust shown by the scientific elite!

3.3.4. Pioneer scientists at the origin of antiseptics, antibiosis, phage therapy and early use of chemotherapeutics (1847-1928)

Antiseptics and disinfectants: Also based on the contributions of Pasteur and Koch, the wide applications of antiseptics materialized (Vandamme, 2018b; Vandamme and Mortelmans, 2020). It had been shown earlier in 1847 by the Hungarian physician Ignaz Semmelweis (1818-1865) that use of chlorine in clinics as hand disinfection for medical personnel could lower infection spread drastically. London physician John Snow (1813-1858) demonstrated that the epidemic outbreak of cholera in 1854 was caused by water supply contaminated with human sewage. In 1865, surgeon Joseph Lister (1827-1912) introduced in the UK hygienic methods in surgery and personal sanitation with great success based on the use of carbolic acid (phenol). It had been in use at a sewage treatment plant in Carlisle to reduce the stench of rotten garbage and that of fields that were irrigated with sewage water. Furthermore, it killed the protozoal parasites of the cattle that grazed on these fields and pastures. Lister's work was at the base of the development of a wide range of antiseptics and disinfectants, chemicals now worldwide in use in medical, industrial and household practice. From the 1880s, a growing awareness of the existence and harmful activities of certain microbes had initiated an obsession among the general public towards increased cleanliness and tidiness in daily life. Until that time common houses were a filthy place to live as were the streets (Ashenburg, 2009; Vandamme and Mortelmans, 2020).

Antibiosis: Early observations on antibiosis can be concluded from folk medicines, practiced over the ages. Since the 1870s documented cases appeared in the scientific literature (Waksman, 1937; Landsberg, 1949; Foster and Raoult, 1974; Selwyn, 1979; Duckett, 1999; Bentley; 2001; Bucci and Galli, 2011). Over thousands of years, moldy bread, cheese and warmed soil were used in folk medicine to heal wounds. In the early 1870's in the UK, John Burdon-Sanderson, John Tyndall and William Roberts observed the antagonistic effects of one microorganism on another (named "antibiosis"), especially that urine samples and culture fluids covered/contaminated with mold did not produce bacteria (Eve and Creasy, 1945; Selwyn, 1979). The very first recorded

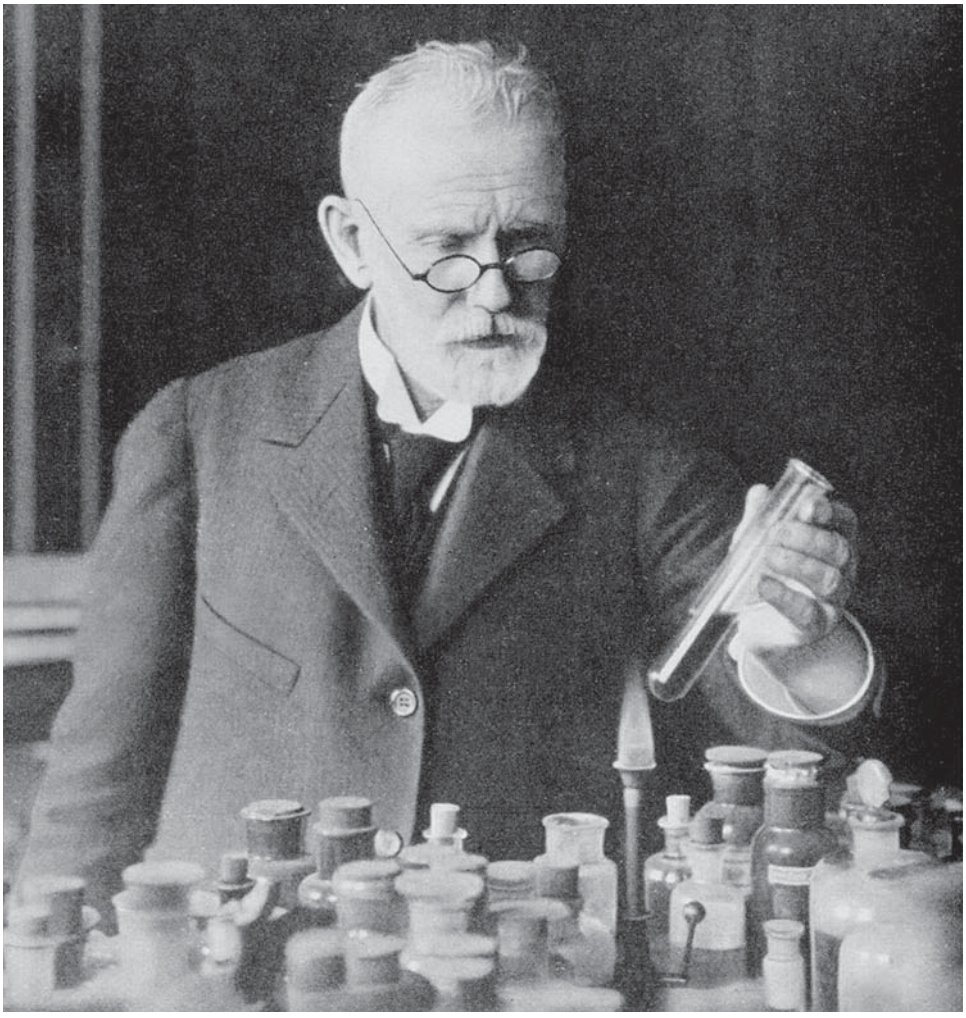


Figure 12. Paul Ehrlich

observation on microbial "antibiosis" dates from 1877, when Louis Pasteur and Jules Francois Joubert described slower growth of *Bacillus anthracis* and *Clostridium* sp. cultures, when contaminated with fungi and other bacteria. With his characteristic foresight, Pasteur suggested that this phenomenon might have some therapeutic potential. In 1895 Vincenzo Tiberio, physician at the University of Naples, Italy, published on a mold from a water well with antibacterial action (Bucci and Galli, 2011). Also in 1895, Italian physician Bartolomeo Gosio, discovered a compound in the culture filtrate of a *Penicillium brevicompactum* fungus, that in crystallized pure form inhibited growth of *Bacillus anthracis*. This compound was later rediscovered and named mycophenolic acid (MPA), but was never used as an antibiotic due to its toxicity. A derivative, MPA-mofetil, discovered in 1995 has been used as a new immunosuppressant (Bentley, 2001). The French army doctor Ernest Duchesne (1874-1912), stationed at the Military Hospital in Lyon, discovered healing properties of a *Penicillium glaucum* mold, even curing guinea pigs of typhoid fever and published his findings in his dissertation in 1897 (Duckett, 1999). In the early 1920's, medical microbiologist André Gratia (1893-1950) and his group at the University of Liége, Belgium, was involved in a concerted effort in the study of the lysis of bacteria by products derived from other microorganisms. His group also observed a fungal contamination, identified as a *Penicillium* species, in one of their *Staphylococcus aureus* cultures that inhibited the growth of the bacteria, and that exerted this action also on anthrax causing bacteria. However, their paper describing this effect received little attention (Gratia and Dath, 1924). Due to an illness, he did not further pursue this research topic and focused later on colicins (Gratia, 2000). In 1928 it was shown that certain lactococcal strains can inhibit the growth of other closely related lactic bacteria, leading to the discovery of the bacteriocins, including nisin now generally in use as a food preservative (Rogers, 1928; De Vuyst and Vandamme, 1994). Over the preceding 30 years various microbial preparations had been tried as medicines, but they were either too toxic, too weak or inactive in live animals (Waksman, 1937; Waksman and Foster, 1937; Dubos, 1939; Landsberg, 1949; Foster and Raoult, 1974; Duckett, 1999). During the following decades landslide discoveries in the domain of "antibiosis" were to come about. Another type of molecules derived from microorganisms, with selective antimicrobial action against other microorganisms was to appear in the coming years, the antibiotics.

Phage-based therapy and control

Bacteriophages (or phages) are viruses that specifically attack, lyse and kill their (host) bacteria. Soon after their discovery in 1915 by Frederick Twort (1877-1950), at the Brown Animal Sanatory Institution, London, UK (Twort,1915) and independently in 1917 by Felix d'Herelle (1873-1949) a French-Canadian Microbiologist at the Institut Pasteur, Paris, France (d'Herelle, 1917), It was realized that they could be used to treat bacterial infectious diseases in a very specific way (Duckworth, 1976). d'Herelle coined the name "bacteriophage" and treated successfully patients with dysentery, caused by *Shigella dysenteriae*, with his phage preparations. He claimed that bacteriophages were very small organisms and this statement met soon with fierce opposition. Belgian 1919 Nobel Prize winner Jules Bordet, a famous immunologist working at the Institut Pasteur in Brussels, Belgium, proposed that the causal agent of bacterial lysis was a normal "autoregulated" secretion of bacteria, an endogenous bacteriolytic enzyme. These scientific disagreements escalated into personal friction with d'Herelle. However, another Belgian group at the Institut de Bactériologie, Catholic University of Louvain, headed by Richard Bruynoghe, defended d'Herelle's view, that phages were ultramicroscopic microorganisms, thereby challenging Nobel Prize winner J. Bordet. This Bordet-Bruynoghe controversy lingered on until the early 1930s, when new technologies and electron microscopy finally came to the rescue, with Bruynoghe and d'Herelle winning this debate (Billiau, 2016; Vandamme and Mortelmans, 2019). Belgian microbiologist André Gratia was also one of the first phage researchers after Felix d'Herelle, though being a supporter of Bordet's views), well before the viral nature of bacteriophages became clear (Gratia, 1921; Billiau, 2016; Vandamme and Mortelmans, 2019). In the 1920s d'Herelle travelled on and off to South-East Asia, India and Egypt, to study and successfully counteract cholera and plague epidemics with his phage preparations. He met in 1934 in Paris Georgian bacteriologist George Eliava (1892-1937), who was the founder of the now Eliava Institute of Bacteriophages, Microbiology and Virology, at the Georgian Academy of Sciences, Tbilisi, in the former Soviet Republic of Georgia, where phage therapy was well developed and successfully practiced all over the Soviet Union and former Eastern-Europe. A range of pathogenic bacteria were cultivated in up to 500-liter scale fermenters that were on purpose "contaminated" in their early log phase with phage solutions, to then harvest the phage lysate liquid and prepare it into "medicinal phage". They collaborated intensively for a few years and phage therapy boomed in prewar times, also driven by the military on both sides (Kutter and Sulakvelidze,2005; Vandamme and Mortelmans, 2019). However, this phage therapy principle was soon (and especially in the West) marginalized by the rise of penicillin, streptomycin, and the subsequent widespread use of antibiotics and chemotherapeutics since the mid 1940s. The now alarming spread of antibiotic-resistant bacteria and the lack of really novel antibiotic compounds in the pipeline demands for an urgent revisit to the potential of phage therapy and phage control of pathogenic or undesirable bacteria by use of their "own" viruses, not only in the medical area, but equally in veterinary practice, crop protection, aquaculture and in the food and sanitation sector (Cabello, 2006; Brussow, 2017; Caselli, 2017). Also, the recent introduction of "magistral phage preparations", phage lysins and the development of engineered and even synthetic phages can reboost the phage therapy concept (Vandamme and Mortelmans, 2019; Pirnay, 2020).

Chemotherapeutics: Towards the end of the 19th century, Koch's pupil and immunochemist Paul Ehrlich began testing many synthetic chemical compounds to treat diseases. His team was successful in 1909, curing relapsing fever, syphilis and trypanosomiasis with an arsenic-based product arsfenamine, named Salvarsan or Compound 606, that killed the syphilis bacterium in vivo without harming the host. This was the first chemical therapeutic drug ever discovered and he coined the term "chemotherapy". Later, in 1912 Ehrlich used synthetic dyes and established the concept of the "magic bullet". This means the use of drugs with selective toxicity to the parasite but not damaging the host. This opened an entirely new field for the curing of human diseases (Dixon, 2006; Williams, 2009). In 1927 this work was continued by bacteriologist Gerhard Domagk (1895-1964) in Germany along with his collaborators Fritz Mietzsch and Josef Klarer at the I.G. Farbenindustrie in Germany. Their work resulted in the development of the red colored chemical, named *Prontosil rubrum*. This compound was active in mice against streptococci but strangely was not active in vitro (Otten, 1986). Then in 1935, chemist Jacques Trefouel (1897-1977) and coworkers at the Institut Pasteur in France discovered that the red dye was hydrolyzed inside

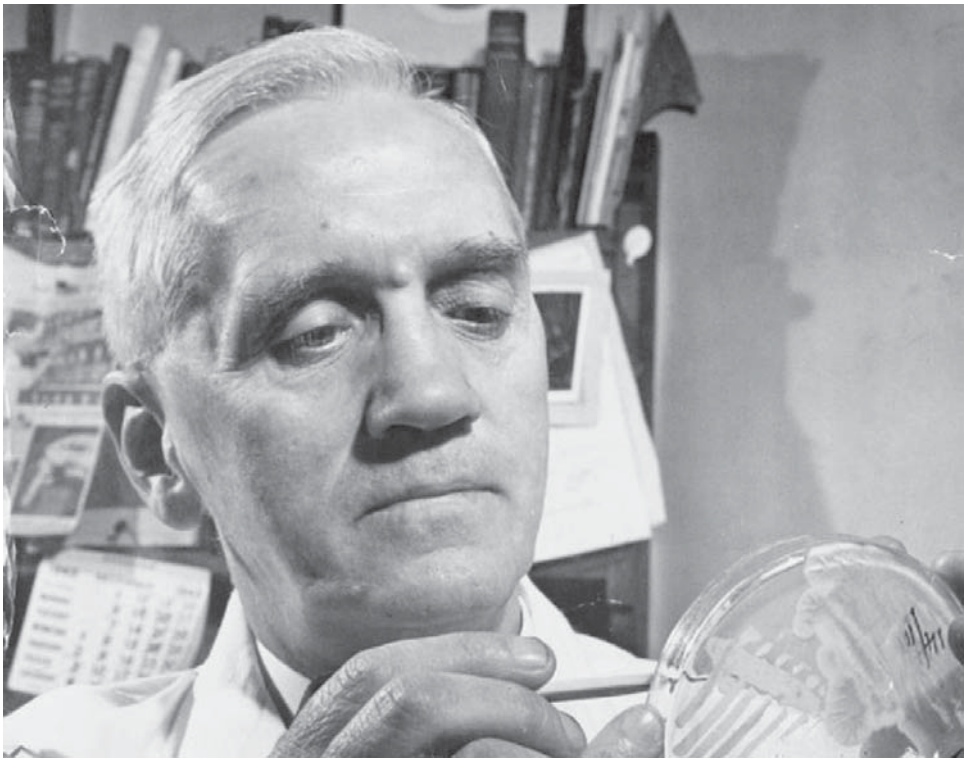


Figure 13: Alexander Fleming.

the animal into the colorless and inhibitory compound sulphanilamide. This established the important concept that chemicals could kill or inhibit bacteria without toxicity to humans. Other synthetic chemotherapeutic drugs gained wide use over the coming years, including isonicotinic acid hydrazide and para-aminosalicylic acid, both to treat tuberculosis. These developments and the introduction of antibiotics in the early 1940s suppressed the then already well developed and widespread use of bacteriophage therapy to treat bacterial infections (Vandamme and Mortelmans, 2019).

3.3.5. The tedious route to the first antibiotic: Fleming's penicillin and its fermentation process: screening, mutation and strain breeding (1928- World War II)

The foregoing precarious on and off research on antibiosis led finally to the momentous moment in microbiological history, when, in September 1928, medical bacteriologist Alexander Fleming (1881-1955) (Figure 13) at St. Mary's Hospital in London, UK, observed in one of his petri dishes a halo of inhibition and lysis of nearby *Staphylococcus aureus* bacterial colony growth close to a contaminant blue-green mold colony, that was then incorrectly identified as *Penicillium rubrum* by mycologist Charles La Touche (1904-1981). It was later identified as a blue-greenish *Penicillium notatum* species, then accepted as *P. chrysogenum* and in 2011 resolved as *P. rubens* (Houbraken et al., 2011). His sharp observation would change history. He also noted that filtrates of the mold lysed the staphylococci and were nontoxic in animals. His earlier discovery in 1921 and studies of lysozyme, led him to recognize this as an important phenomenon to pursue. He coined the name penicillin in March 1929 for the antibacterial substance in the mold culture broth and published his findings (Fleming, 1929), but he was unable to isolate and purify the compound in sufficient quantities for further studies (Brown, 2004; Lax, 2005). Since the activity was very unstable and Fleming could get no encouragement from his fellow scientists concerning the usefulness of such material, the project was abandoned by Fleming and interest waned. In the early 1930's, British chemist Harold Raistrick (1890-1971) and his colleagues at the London School of Hygiene and Tropical Medicine tried to isolate penicillin but the instability of the substance also frustrated their efforts (Clutterbuck et al., 1932). For a decade penicillin remained as a laboratory curiosity. Although Fleming's discovery finally led to penicillin, the first successful chemotherapeutic agent produced by a microbe, thus initiating the golden age of the antibiotic wonder drugs, the road to the development of penicillin as a successful drug was not an easy one (Abraham, 1990; Brown, 2004; Lax, 2005). It took another 15 hectic years before penicillin lived up to its expectations and was produced by fermentation on industrial scale, a process still going strong up till today with over 100 antibiotic compounds in use. However, the current worldwide spreading of general antibiotic resistance due to over-prescription and nonclinical use overshadows this success story (Cabello, 2006; Brussow, 2017; Caselli, 2017). In his Nobel-lecture in 1945, Fleming already had warned of the danger of antibiotic resistance!

It was not until the end of 1938 that Howard W. Florey, Ernst B. Chain, Norman G. Heatley, Edward P. Abraham and their colleagues (the Oxford team) at the Sir William Dunn School of Pathology, at Oxford University, UK, took up interest in penicillin again as an example of antibiosis and possible therapeutic activity. This research followed E.B. Chain (1906-1979), a Berlin born Jew, who fled Nazi Germany to the UK in 1933. There he searched through Fleming's and others' papers on lysozyme, penicillin and other metabolites with inhibitory effects on other organisms. It is speculated that the persecution of Jews and the threatening annexation of Austria that very year by the German Nazis, as well as the general war atmosphere in Europe also had a role in the selection of these topics. In 1939 René Dubos (1901-1982), a former student of Prof. Selman A. Waksman (1888-1973), a soil microbiologist and biochemist at Rutgers University, NJ, USA, announced the discovery of an antibiotic complex tyrothricin, based on screening of soil microbes, while at the Rockefeller Institute of Medical Research, New York, USA (Dubos, 1939). A few years earlier Waksman had hypothesized that numerous soil microbes, especially actinomycetes were able to produce antimicrobial metabolites that enabled a form of antimicrobial warfare in the soil (Waksman, 1937). The discovery of tyrothricin and Chain's group 1940-publication showing the clinical potential of penicillin as a systemic drug stimulated Waksman to intensify his efforts to screen Actinomycetes filamentous soil bacteria for antimicrobial metabolites (Chain et al., 1940). Waksman's group discovered in the following years actinomycin, streptothricin and in 1943, together with his postgraduate Albert Schatz (1920-2005), streptomycin, the first drug that successfully treated tuberculosis. It was indeed shown that soil microbes were able to kill medically important pathogenic bacteria (Schatz et al., 1944). Schatz soon became convinced that he had been the victim of an injustice, in that Waksman was minimizing his role in the streptomycin discovery and taking all the credit for their joint achievement. He left the Rutgers soil microbiology department bitterly in 1946 and sued in 1950 Waksman and the Rutgers Research and Endowment Foundation for a share of the royalties and recognition of his role in the discovery of streptomycin; an out-of-court settlement was eventually reached.

In the years 1939-1941, under eminent war pressure, Fleming's penicillin producing strain classified as *Penicillium notatum* NRRL 1249 was grown by the Oxford team as surface culture in unshaken larger lab-flasks, Fernbach flasks, bedpan types and milk bottles. Fermentation media were also optimized. Penicillin activity measurements were improved and quantified "arbitrarily" by Norman G. Heatley (1911-2004), a keen and inventive assistant to Florey, who developed the "Oxford Cup Method" to define the Oxford Unit (OU). It was not until October 1944 that an international unit (IU) based upon weight was established, namely 1 mg of pure penicillin was equal to 1.667 international Oxford units or 1 international unit equals 0.6 µg. Solvent extraction of penicillin from the broth was optimized and freeze drying was used to obtain penicillin in a dry powder form. Toxicity tests in animals (mice) were also conducted. Mice injected with lethal doses of virulent *Streptococcus* sp. survived, while all controls died. These amazing efforts led to the successful preparation of a stable form of penicillin and the demonstration of its remarkable antibacterial activity and lack of toxicity in mice. Subsequent clinical trials with purified penicillin on humans were very successful and time had come for commercial production. Production of penicillin by the original strain of "*P. notatum*" in use was so slow however that it took over a year to accumulate enough material for a clinical test on humans. Large-scale production became essential. Since British pharmaceutical companies (Wellcome, Boots and Imperial Chemical Industries) did not show interest, the Oxford University administration was forced to contact their funding organization, the Rockefeller Foundation in New York, USA. Florey and Heatley were sent to New York, where they arrived on July 3, 1941, and visited some pharmaceutical firms in the region, however with little to no interest shown. They met on July 9 with R.G. Harrison, the chairman of the National Research Council in Washington D.C., who informed them to contact the Department of Agriculture in Washington D.C., where they met Percy A. Wells, acting chief of the Bureau of Agriculture and Industrial Chemistry. He was in charge of the four regional research laboratories and fortunately was a fermentation specialist! It was Wells, advised by Charles Thom, a world authority on *Penicillium* molds who sent Florey and Heatley to the Agriculture Department's Northern Regional Research Laboratories (NRRL) in Peoria, Illinois, where they arrived on July 14 with the historical outcome as a result! Florey and Heatley convinced the NRRL and several American pharmaceutical companies

(Merck and Co. as a first, and later on Squibb, Lederle and Pfizer) to develop large-scale fermentation production of penicillin (Bennett et al., 2020). Heatley remained until September 1941 at NRRL to work with Robert D. Coghill, head of the Fermentation Division, and then worked for a while at Merck and Co., passing the Oxford knowledge (culture media components such as Brewer's Extract, assay of penicillin, and extraction procedures) for free to both locations (Foster and Woodruff, 1943; Coghill, 1944; Herion, 2000). By the end of 1941 yields of 40 OU were obtained in surface culture and selection of monoconidial cultures from NRRL 1249 yielded strain 1249.B21 that produced up to 200 OU of penicillin F (2-pentenylpenicillin). These levels raised the interest to the pharmaceutical industry. However, these strains did not produce those high levels in shaken cultures or submerged fermentations, a technique that was imperative for large scale fermentation tank production. One 50.000-liter culture tank was the equivalent to 70.000 milk bottles, allowing huge savings in labor and handling. A search in the NRRL culture collection led to strain *P. notatum* NRRL 832, that produced only up to 50 OU but of the more desirable penicillin G (benzylpenicillin) under submerged culture conditions. This strain originated from Philibert M. Biourge's lab collection, a famous mycologist at the Catholic University of Louvain, Belgium. This NRRL 832 strain was initially used for over a year for bulk penicillin production in submerged fermentation tanks but attempts for higher yields than 100 OU by selecting better variants were not successful. Thus began a momentous cooperative effort among university and industrial laboratories in the United States and academic institutions in England which lasted throughout World War II, as part of the US Government War Production Board to rapidly commercialize penicillin to meet the needs of this life-saving drug (Abraham, 1990; Brown, 2004; Lax, 2005). In early 1942 the technical director of Chester County Mushroom Labs. in West Chester, PA, G. Raymond Rettew also contacted the Department of Agriculture and was referred to the Office of Scientific Research and Development (OSRD) with his expertise on culturing mushrooms and fungi (on solid media) and with his conviction that his firm had already the equipment and knowledge to be able to produce penicillin quickly. The OSRD agreed and his small company became a collaborator in the overall penicillin project. He affiliated with Wyeth Laboratories Inc., a Philadelphia pharmaceutical company owned by American Home Products Corporation and was asked by the War Production Board to produce penicillin by the then established surface culture method, while other firms still had to develop the submerged deep fermentation culture system. Wyeth supplied their first penicillin to the US Government in June 1943. However, by 1945 most companies, including Wyeth, were using the more efficient deep culture fermentation process. Initially NRRL researchers experimented with rotating drums as a submerged culture system, that later evolved into static tanks with sterile air pumped through the culture broth with rotating agitation blades and cooling coils. In those first years Coghill's collaborator Andrew J. Moyer replaced the not readily available Brewer's Extract by corn steep liquor (CSL), a cheap byproduct of the cornstarch processing industry. It was found to boost the penicillin yield up to 200 IU/ml. Scientists at NRRL and Corn Products Refining Co. demonstrated that this effect was due to the presence of phenylacetic acid in CSL, shown later to be a precursor molecule for benzylpenicillin or penicillin G. Also, lactose was found to be a better carbon source than glucose for penicillin production (Moyer and Coghill, 1947; Raper, 1978, 1994).

A cooperative "strain-selection" program was established between researchers at the USDA-NRRL in Peoria, at Pennsylvania State University, the Carnegie Institute at Cold Spring Harbor in New York, University of Minnesota, Stanford University and at the University of Wisconsin-Madison. Strain selection began with an appeal for samples of soil, moldy grain, fruits, and vegetables, sent in during 1942 and 1943 from anywhere in the USA, but also by Navy and Air Force members worldwide. A young lady known as "Moldy Mary" was employed to scan the local markets, shops, bakeries and other places in Peoria for samples showing greenish mold. Contrary to favorite anecdotes is that the moldy cantaloupe that finally yielded the bonanza strain, identified and named as *Penicillium chrysogenum* NRRL-1951, was brought to NRRL by an attentive Peoria housewife. Those strains were screened against NRRL 832 and NRRL 1249.B21 as controls. This bonanza strain was capable of producing 60 µg (or 100 IU) per ml of culture broth, comparable to NRRL 832. Cultivation of spontaneous sector mutants and single spore isolations led to higher-producing cultures of NRRL-1951, both in surface and submerged

culture. One of these, NRRL 1951-B25, produced up to 150 µg per ml (or 250 OU/ml). This strain was sent to the collaborating laboratories and companies in April and May 1944. Conidia of this strain were subjected to X-ray treatment by Milislav Demerec of the Carnegie Institute at Cold Spring Harbor. Survivor-strains were sent to the University of Minnesota for comparison and sent then to the University of Wisconsin for further shake flask-testing by Elisabeth McCoy's team at the Dept. Bacteriology, that forwarded the best ones to W.H. Peterson and Marvin Johnson of the Department of Biochemistry for small tank fermentations. One strain X-1612 was superior, yielding over 450 to 500 OU/ml (300 µg per ml). This was confirmed at NRRL in Peoria in 60-gallon fermenters on corn based-lactose media. Strain X-1612 was then in turn subjected to UV irradiation by M.P. Backus and J.F. Stauffer at the Botany Department of Wisconsin University. One of these surviving strains, named Q-176, produced up to 900 OU/ml (550 µg per ml) on the improved culture media. However, it turned out that not penicillin G was formed but the less stable penicillin K. Fortunately at that time it was known that phenylacetic acid was part of the penicillin G molecule and by adding this compound -as a precursor to the culture medium the desired penicillin G was formed at the same level. The Q-176 strain became the ancestor of all of the strains subsequently used in industry. The "Wisconsin family" of superior strains, obtained by consecutive exposures to UV or mustard gas and by selection of chrysogenin pigmentless mutants which facilitated purification, became well known all over the world, some producing over 1800 µg per ml. The strains were not patented and given freely to private industries that further improved the process to yield far over 50.000 OU/ml (30 mg/ml).

Although Fleming's original strain produced only traces of penicillin on surface culture media (2-4 IU/ml), "brute force" genetic manipulation made tremendous strides in production ability and led to a whole new technology known as "strain improvement" or "strain breeding". These early basic genetic studies concentrated heavily on the production of *Penicillium* mutants and the study of their properties. The ease with which "permanent" characteristics of microorganisms could be changed by mutation and the simplicity of the mutation technique had tremendous appeal to microbiologists (Raper,1994).

The penicillin improvement effort was the start of a long engagement between genetics and industrial microbiology which ultimately demonstrated that mutation was the major factor involved in the hundred to thousandfold increases obtained in production levels of many other microbial metabolites (Prescott and Dunn, 1959; Raper,1978, 1994; Elander, 2002; Baltz et al., 2017).

4. Epilogue: from Bacchus to Synthia

As outlined above, the practice of fermentation processes and industrial microbiology has its roots deep in antiquity and microorganisms were unknowingly exploited to serve the needs and desires of humans. The current impact of relevant areas of research and applications in fermentation science, industrial microbiology and industrial biotechnology is very broad and is now of utmost importance for the overall wellbeing of people and the planet in providing a green technology, based on the use of renewable resources. The term "biotechnology" was coined already in 1919 by a Hungarian agricultural engineer Karoly Ereky (1878-1952) and referred indeed to processes, whereby "raw agro materials, now called renewables, could be biologically or biochemically upgraded into socially useful products" (Ereky, 1919; Fari and Kralovansky, 2008). However, his new term was hardly used and almost forgotten till the mid 1970s, when it came really in fashion and lived up to its promises in science and technology (Bud, 1993; Baltz et al., 2010, 2017)! Numerous invaluable bulk and fine chemicals, pharmaceuticals, peptides, antimicrobials, amino acids, vitamins, conventional and mRNA vaccines (Weissman and Kariko, 2015), biopolymers, specialty sugars, enzymes, rDNA peptides, monoclonal antibodies (MABs), growth factors, analytical kits and reagents that are all based on these biotechnologies are now on the market (Baltz et al., 2010). The development and introduction of contained rDNA fermentation technology in the early 1970s has been crucial in providing a boost to the field that lasts up till today. The construction of the "artificial" microbe named "Synthia" (*Mycoplasma laboratorium*) in early 2010 at the J. Craig Venter Institute, CA, USA, demonstrated the potential and controversy of the new biotechnologies (Gibson et al, 2010; Leak, 2010).

In retrospect all these recent developments are based on, and inspired by, the fundaments gradually provided over

time by the early pioneers and scholars of microbiology, fermentation, industrial microbiology and biotechnology. Jackson W. Foster (1914-1966), penicillin fermentation-microbiologist at Merck &Co. Inc., Res. Labs, N.J, USA, stated in the 1940's: "Never underestimate the power of the microbe" (Foster and Woodruff, 1943). And Louis Pasteur said about 150 years ago "Messieurs, c'est les microbes qui auront le dernier mot (in English: Gentlemen, it is the microbes that will have the last word). Microbes themselves remain invisible and invincible and they are so powerful as reflected both in numerous beneficial "fermentations" (Baltz et al., 2010, 2017; Soetaert and Vandamme, 2009, 2010) and harmful actions (Sherman, 2007; Vandamme and Mortelmans, 2020), as history has proven over the ages and through to today. Both these "old" statements reflect this microbial power that is still going strong!

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SIMB regrets to announce postponement of the November 7–10, 2021, RAFT® meeting and premeeting workshops and their rescheduling for November 6–9, 2022. The ongoing COVD-19 pandemic and recent Delta variant, nationwide bans on nonessential US travel as well as international travel bans have made it impossible for attendees to participate in and attend RAFT® 2021.

The meeting will return to the Hyatt Regency Coconut Point Hotel. Dates for opening the abstract site, registration and exhibits will be available after the new year.

Registration refunds will be issued and we appreciate your patience during the process.

For information on exhibit table refunds and exhibitor showcases, contact *tina.hockaday@simbhq.org* or *meetings@simbhq.org*.

Photo by Ariel on Unsplash

2020 & 2021 SIMB Annual Meeting Awardees

SIMB is pleased to announce the 2021 awardees whose were presented at the August SIMB Annual Meeting in Austin, Texas.

2021 Charles Thom Award
Lee Lynd, Dartmouth

2021 Charles Porter Award
Nigel Mouncey, Joint Genome Institute

2021 SIMB Fellow
Thomas Klasson USDA

2021 Waksman Outstanding Teaching Award
Sidney Crow, Georgia State University

2021 Young Investigator
Marc Chevrette, University of Wisconsin

Also recognized during the Annual Meeting in Austin were the 2020 SIMB Awardees:

2020 Charles Thom Award
Nancy Keller, University of Wisconsin

2020 Charles Porter Award
Debbie Yaver, Yaver Biotech Consulting

2020 SIMB Fellows
Krishna Madduri, Nicholls University and Jonathan Mielenz, White Cliffs Biotechnology Consulting

2020 Waksman Outstanding Teaching Award
Rajesh Sani, South Dakota School of Mines

2020 Young Investigator
Kang Zhou, University of Singapore

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Call for Abstracts opens October 2021

Session Topics

Topic area 1 Biofuels, Bioproducts, and Synthetic biology Topics of interest involve lignocellulose (sugars and lignin) upgrading to fuels, chemicals and material precursors via chemical and biological tools. The reincorporation of ethanol in the product portfolio for the generation of jet fuel is also encouraged. Algae.

Topic area 2 Alternative feedstocks and biomaterials- Topics of interest are plastics deconstruction and upcycling, waste valorization, CO2 sequestration and metabolism of C1, and biomaterials among others

Topic area 3 Biomass engineering and deconstruction – Topics of interest involve bioenergy crops and plant genetics, biomass deconstruction and fractionation, biomass degrading enzymes, and cell free systems among others.

by Elisabeth Elder

Legionellosis Diagnosis and Control in the Genomic Era

Jacob Moran-Gilad and Rachel E. Gibbs (Editors)
ISBN: 978-1-913652-53-1 (paperback); 978-1-913652-54-8 (ebook)
2020
Caister Academic Press, UK

Legionellosis Diagnosis and Control in the Genomic Era was edited by Jacob Moran-Gilad and Rachel E. Gibbs who are both associated with Ben Gurion University of the Negev in Beer Sheva, Israel. Before starting the review of the book, I should let you know that reading it brought back lots of memories. *Legionella pneumophila* was one of the organisms I studied in working with development and control of biofilms using organic N-halamine disinfectants that were being developed by S. Davis Worley in the Department of Chemistry at Auburn University. Several chapters in the book reminded me of the challenges involved in culturing the organism as well as places from which it can be isolated and the health issues in which it is involved. While my work was limited to *L. pneumophila*, the current book is more extensive in the species as well as serogroups covered.

The editors contributed the first chapter of the book which provides a brief overview of the *Legionella* genus by reminding the readers that the organism was first discovered in 1977 and points out its characteristics of being isolated from many environments, its abilities to colonize humans as well as protozoans, and its resistance to antibiotics. They also point out that the domain has been revolutionized by genomic methods.

Chapter 2, written by Rafael Garduño of Dalhousie University in Halifax, Nova Scotia, covers the interactions of *L. pneumophila* with freshwater amoebae plus biotic and abiotic components of freshwater environments. Garduño also discusses the pleomorphic developmental forms of *L. pneumophila* and the ability of *L. pneumophila* and *Legionella longbeachae* to colonize soils.

Chapter 3 was written by Elisabeth Kay, Virginie Lelogeais, Sophie Jarraud, Christophe Gilbert, and Patricia Doublet who represent the Centre International de Recherche en Infectiologie in Lyon, France; and the Centre de Biologie et de Pathologie Est in Bron Cedex, France. These authors describe the biological features used by *L. pneumophila* to invade, survive within, and control, host cells including human alveolar macrophages as well as protozoa by relying on secretion systems. In addition, this chapter covers the importance of secreted proteins and genes which are involved in environmental interactions and virulence.

Chapter 4 was written by Natalia A. Kozak-Muiznieks, Jeffrey W. Mercante, and Brian H. Raphael who represent the Centers for Disease Control and Prevention in Atlanta, GA, USA. This chapter introduces the historical information on the reference strains of *L. pneumophila* along with clinical and epidemiological information.

Chapters 5 and 6 were written by Giancario Ceccarelli, Mario Venditti, Maria Scaturro, and Maria Luisa Ricci who represent the University of Rome and the National Institute of Health in Rome. Chapter 5 covers clinical information on Legionnaires Disease and Pontiac Fever including diagnosis, treatments, and impacts of immune system problems. Chapter 6 covers the importance of laboratory diagnostics as well as their development and their shortcomings.

Chapter 7 was written by Diane S. J. Lindsay who represents the Scottish Microbiology Reference Laboratory in Glasgow, UK. This chapter is similar to the previous two with the exception that it covers non-*L. pneumophila* species in the genus *Legionella*.

Susanne Surman-Lee and James T. Walker, authors for Chapter 8, represent Leegionella Ltd., Rockford, Ringwood BH24 3NA, UK, a British Public Health Microbiology Consultancy and Advisory Services and Walker on Water, UK (*www.walkeronwater.org*). This chapter covers prevention, risk assessment, and risk management of *Legionella* outbreaks.

Chapter 9 was written by Birgitta de Jong and Lara Payne Hallström who represent the Centers for Disease Control and Prevention in Sonia, Sweden. This chapter covers the systematic European approach to the surveillance methods in the European Union.

The authors of Chapter 10 Norman K. Fry and Sophie Jarrud represent the Public Health England – National Infection Service, London, UK; the Institute for Infectious Agents, Lyon, France; and Equipe Pathogénèse des Légionelles, Lyon, France. This chapter adds additional coverage to genomic diagnosis of *Legionella*.

The final chapter, written by Daniel Wüthrich, Helena M. B. Seth-Smith, and Adrian Egli who represent the University Hospital, the University of Basel, and the Swiss Institute for Bioinformatics, all of which are in Basel, Switzerland. This chapter covers typing of *Legionella* isolates using phylogenetic analysis, next generation sequencing and genomics.

The chapters are well organized, easily read, and comprehensive. The book will be of interest to clinical microbiologists, epidemiologists, geneticists, environmental microbiologists, and historical microbiologists who are in advanced courses of study or are faculty members around the world.

Upcoming SIMB Meetings

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San Francisco Hyatt Regency • San Francisco, CA

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