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Peoria, An International Center of Fermentation Excellence*

C.W. Hesseltine
5407 Isabell
Peoria, IL 61614

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I wish to develop the thesis that the Peoria area was and is a center of worldwide excellence in fermentation.

First Distilleries

As far as I can tell, the first distillery in Peoria was started by an enterprising man named Almiran S. Cole in 1844. People believed that this enterprise would end in failure because of lack of money and the fact this was a different type of work for Peoria, plus the presence of temperance societies. Factors that favored this business was the low price of corn and the relatively high price of whiskey. Cole's steam distillery was capable of using 200 bushels of corn per day. In spite of the fact that by the time he started up, he was almost broke and his creditors were closing in on him, the plant began to immediately make money. People saw that Cole was getting rich and a Mr. Thompson bought him out. Cole then, with money and experience, immediately built a 3-times larger plant. It soon was apparent that substantial sums of money could be made and by the year 1864, 12 distilleries were in operation using from 300 to 1200 bushels of corn with a total of 10,500 bushels of grain per day. The residual grain left after the alcohol was extracted was sold for feeding cattle and pigs. Disaster struck in this year when the government assessed a 2-dollar-per-gallon tax on whiskey made after 1864. Those companies with large inventories of whiskey made fortunes in the 2-dollar-a-gallon differential. The above information was obtained from Ballance's History of Peoria 1870, which devotes a whole chapter on distilleries in Peoria.

Hiram Walker (1816-1899)

The Hiram Walker Company for many years operated the world's largest bourbon whiskey plant in Peoria. Mr. Walker was a remarkable man who, in 1856, invested \$40,000 in the milling and distilling business. Although he was an American, because of the unstable temperance laws in Michigan, he built his first plant in Canada in 1856. At this time whiskey was sold in barrels in grocery stores without any manufacturers' names. Walker, knowing he had a superior product, began packaging his whiskey in small barrels with his name on them. He also introduced the sale of whiskey in large earthenware jugs and in bottles that were sealed. Other distilleries soon had to identify their product to compete.

The company anticipated the end of prohibition, and when its repeal came in 1933, the company had already started to build a plant in Peoria which began production shortly after repeal. About 1890 a remarkable man appeared on the scene in Peoria whose discoveries were revolutionary and whose processes are still widely used.

Takamine

Dr. Jokichi Takamine was the father of commercial enzymology and one of the pioneers of biotechnology. He was born in 1854 in Japan and received his Doctor of Pharmacology and Ph.D. in Engineering. He became very interested in Western concepts that would be useful to the Japanese but he also was

interested in Japanese contributions to the West in science, industry and culture. In 1884 he married an American girl whose father helped him financially start the Takamine Ferment Company in Peoria in 1890. This company's business was to produce "diastase," a mixture of glucoamylase and α -amylase. This mixture of amylases was produced by the koji molds (*Aspergillus oryzae* and *A. soyae*) and it was here in Peoria that fungal amylase was first introduced to the West. In the production of alcohol from grain, it is necessary to break down the starch to sugar, which is then fermented to alcohol. Up to this time the starch conversion step of grain was brought about by the use of malt produced by the germination of grains.

In Takamine's process the malt was replaced with fungal α -amylase and, therefore, was a process in direct competition with malt utilization. Apparently this new innovation was greeted with apprehension by the malt manufacturers. There was labor agitation and a propaganda campaign against Takamine. In 1894 the distillery where he worked was burned to the ground. Arson was suspected but never proven. The distillery which used fungal amylase was rebuilt, but the company dissolved the Takamine corporation and Takamine left Peoria in 1894. In 1894 Takamine obtained a U.S. patent on his enzyme diastase preparation called Taka-Diastase.

Later Takamine founded the Takamine Laboratories in the East and the Sankyo Pharmaceutical Company of Tokyo. He also isolated crystallized adrenaline at the same time that this was done by J. Abel at John Hopkins. Takamine obtained patents on adrenaline. He is known also for arranging for three thousand cherry trees for planting at the Tidal Basin in Washington. Recently a book on Dr. Takamine was prepared and printed by the Miles Company, which some years ago had bought the Takamine Laboratories. The book contains his writings translated into English along side the original Japanese.

Northern Regional Research Center

The Northern Regional Research Center (NRRC) when it was started, had the responsibility for industrial fermentation development. It is significant that the first Director was Orville E. May, a man who had worked in the fermentation field and had published on such fermentations as gluconic, lactic, and koji acids along with the development of rotary aluminum fermentors. He later went to Washington D.C. and subsequently became Chief of the Bureau of Agriculture and Industrial Chemistry. The second Director, H. T. Herrick, likewise had experience in the fermentation process used to convert tannin to gallic acid using *Aspergillus*. He proposed that the Center concentrate on mold fermentation. He, in turn, later went to Washington, D.C. The Chief of the Fermentation Laboratory was Dr. Robert Coghill, a former Professor of Organic Chemistry at Yale University. This laboratory consisted of the Culture Collection (Kenneth B. Raper), Chemical Section (Frank Stodola) and the Survey and Development Section (George Ward).

With the concentration of people with fermentation backgrounds, both at Peoria and in Washington, D.C., it was predictable that Peoria would be the center of fermentation excellence in the United States, if not in the world. The wisdom of this organization was soon to be justified. The concentration of people in research teams and the appointment of administrators with laboratory knowledge ensured success. Today we should remember what history has taught us. Be sure the driver knows how to drive!

The first Chairman of the Peoria Section of the American Chemical Society was Dr. L. B. Howard of NRRC.

Penicillin - Moyer

Penicillin and its effectiveness as an antibacterial drug was discovered in England before World War II. Even though it was known to be a very antibacterial agent that would save thousands of lives during the war, little could be produced because no industrial process was known that could be used to produce the quantities needed. Its synthesis was too difficult. The problem of growing the mold and producing penicillin was brought to the Fermentation Laboratory of the Department of Agriculture at Peoria in 1941. Dr. Andrew Moyer and others were assigned the problem of developing a practical method of making penicillin by fermentation. The team began work in July 1941 and by November 1941 had increased the yield of penicillin tenfold. Dr. Moyer was a mycologist and an expert on industrial mold fermentations. In the first few months he discovered that corn steep liquor, a waste product from the wet corn-milling industry, produced a great increase in penicillin even though it had never been used to grow microorganisms before. Dr. Moyer also discovered that if lactose, milk sugar, was incorporated in the corn steep medium, further increase in penicillin yields resulted.

Another great step forward was the discovery by Dr. Moyer that certain strains of *Penicillium chrysogenum*, isolated by Dr. K. Raper, could be grown in liquid media with continuous agitation or shaking. With these conditions and with special mold strains, commercial quantities of penicillin could be produced. The earlier ineffective method of penicillin production was to seed the mold on the surface of liquid medium where the mold slowly grew and produced small amounts of penicillin. This required thousands of milk bottles. Often contamination was a problem and very low yields were the rule. For example, it took 70,000 bottles to produce the same amount of penicillin as one 10,000 gallon tank could produce using agitation. By 1945 American companies using Moyer's methods had reached the astounding production of 14,000 pounds of penicillin as compared to none in 1941.

The ability to produce a useful drug by fermentation revolutionized chemotherapy. Prior to the penicillin work, antibacterials were organic compounds. NRRC's work showed that a mold antibacterial agent could be produced commercially by submerged fermentation methods. The result of this success was to change the whole direction of searching for new antimicrobial

agents. Many new antibiotics were found. Even to this day corn steep liquor is used in the production of many antibiotics and all, as far as I know, incorporate the use of agitated liquid media in fermentation processes as discovered at NRRC.

The value of penicillin and other antibiotics totals billions of dollars and accounts for the saving of countless lives. This pioneering work resulted in the development of a whole new billion-dollar industry that still utilizes the discoveries of Dr. Moyer.

Dr. Moyer grew up as an orphan and had to struggle to obtain an education. As a result of his childhood struggle, he was always rather suspicious of others. As a scientist he was a careful worker who spent a great deal of time planning his research and rechecking and evaluating his data. Because of his poor background he was not a good speaker or writer, but this was compensated by his clear thinking and his dedication and enthusiasm for research on mold metabolites.

As a result of this work, Dr. Moyer, in 1987, was honored by being posthumously elected to the Inventor's Hall of Fame, 28 years after his death. Only two microbiologists, Louis Pasteur and Moyer, have ever been elected to the Inventor's Hall of Fame. One of the requirements to be elected is that the person honored hold patents that are the basis of a whole new industry; thus, people such as Edison, Bell and the Wright Brothers are in the Hall of Fame. At the time of Moyer's election, 3 other people were selected, namely, W. S. Burroughs (adding machine), Beckman (pH meter) and Sikorsky (helicopter). As far as I know, Moyer is the only government employee ever to be selected. At the time of Moyer's election only 44 people had been honored. When he retired in the early 1950's he held only a GS-12 rating in the government service.

Penicillin - Others

Other individuals involved in the penicillin effort who are not alive should be named. One of these was Dr. Kenneth Raper who isolated improved penicillin producing strains, which gave both higher yields and which would grow in submerged culture. Another person was Dr. Charles Thom who was instrumental in getting the fermentation research placed at NRRC and who was the world's expert at this time on the classification, biology and ecology of *Penicillium*. Both were recognized as outstanding mycologists, and both were elected to the National Academy of Science. Three microbiologists from Peoria have been elected presidents of the Society of Industrial Microbiology and the Mycological Society of America. The penicillin team received numerous awards including the Lasker Group Award of the American Public Health Association in 1946.

ARS Culture Collection

One of the unique fermentation features in Peoria is the collection of living microorganisms maintained at the Northern Regional Research Center (NRRC) of the U.S. Department of Agriculture. The ARS Culture Collection is only one of

two large culture collections in the U.S., and its primary function is to find and maintain useful or potentially useful microbial genetic and taxonomic material.

This collection dates back to 1904 when Charles Thom was hired by USDA to investigate the mold-cheeses. In making these studies Thom secured several hundred strains of *Penicillium* and *Aspergillus* which became the nucleus of the ARS Culture Collection. At first Thom was at the Connecticut Experiment Station but in 1913 he moved to Washington, D.C. taking his cultures with him. The oldest mold culture in the Collection is a strain of *Actinomucor elegans* isolated in 1892. Thom's collection had no formal recognition, and the story is told how Thom worked in the laboratory on weekends to transfer his collection. In 1940 when the Fermentation Laboratory was established at NRRC, the Thom Collection was moved from Washington, D.C. to Peoria and was formally recognized as one of the fermentation groups with Dr. K. Raper in charge. Dr. J. Wickerham joined the group to be curator of the Yeast Collection, and W. C. Haynes was selected to maintain a Bacterial Collection. Since then the number of curators have increased from 3 to 6. Some famous private collections were added in 1940, including the Harvard Collection which had strains isolated before 1900. The yeast collection nucleus was composed of the many yeast cultures brought by Dr. Wickerham from the University of Illinois. Besides this valuable collection of yeasts, Wickerham developed a new technique for the preservation of microbial cultures with the lyophilization process. This technique was used for the entire culture collection and was adopted on a worldwide basis. It made possible the assembling of large collections of microorganisms which could be preserved for years under refrigeration, thus reducing the number of generations and minimizing the possibility of spontaneous mutation.

This process of preservation has now made it possible for the collection to maintain over 80,000 pure cultures of yeast, bacteria, and fungi and maintain almost all strains of microorganisms that produce useful products by fermentation.

In the late 1940's the U.S. Patent Office decided that to make a fermentation patent operable the organism must be available to the public. This meant that a producing strain, not necessarily the best producer, had to be deposited in a permanent culture collection which would make cultures available to the public. The practice of depositing microorganisms in culture collections began in 1949 with the deposit of *Streptomyces aureofaciens* by Lederle Laboratories as NRRL 2209 in the ARS Culture Collection. This deposit was in connection with the fermentation production of aureomycin and the procedure established by NRRC for depositing cultures has been followed ever since. Today, only two U.S. Collections are recognized as suitable places for depositing cultures to satisfy the U.S. Patent Office requirements. These are the ARS Culture Collection and the American Type Culture Collection. This patent law practice has now become established on a worldwide basis.

Cultures are made available upon request at no cost except that there is a deposit charge for patent deposits and a charge for securing patent deposit

cultures. In addition to the extensive taxonomic studies on various industrial groups of microorganisms, the curators recently established methods based on nuclear DNA and ribosomal RNA similarities for solving difficult classification problems. These new techniques offer solutions to problems of taxa which cannot be resolved with conventional taxonomic methods. These new molecular approaches have attracted persons from all over the world to learn the techniques at NRRC.

The outstanding reputation of the ARS Culture Collection is, in part, due to the fact that each curator has a research project on the group of microorganisms he maintains and assists with other projects in biotechnology.

Polysaccharides

For many years polysaccharides, produced by bacteria and yeasts have been investigated as to their chemical structure, their fermentation production, their classification and their physiological effect on animals. Two commercialized products developed by the Center are dextran and xanthan gum. Another group of polysaccharides, the phosphomannans, are of great interest because of their medical implications.

Polysaccharides of microorganisms are of three types with respect to their cellular location, namely intracellular, cell wall and exocellular. The last type is preferred in production because the cells can be separated easily from the polysaccharide.

As a result of the need for a blood-plasma volume-expander during the Korean War, a search was made for a microbial polysaccharide that could do this. Dextran, as produced by *Leuconostoc mesenteroides* NRRL B-512F, a strain isolated from a spoiled can of soft drink, was selected and is used commercially. Now there is an enormous amount of literature on the immunochemistry of NRRL dextrans. These studies have contributed significantly to understanding reactions with antibodies and with animal and plant lectins.

Xanthan gum, on the other hand, was discovered in a survey of microbial gums with no specific activity in mind. Although *Xanthomonas campestris* was known to produce polysaccharides, no information existed on their properties or their chemistry. The polysaccharide, xanthan, was recognized as having great industrial potential because of its unique properties including being stable at high temperatures and acid pH's, colorless, not contributing calories to food, viscous at low concentrations, yielding high viscosities and is resistant to shear-thinning. FDA approved its use as a thickening agent in foods: and today it is widely used in many foods. A second industrial use, in its crude form, is as a petroleum recovery agent. A third class of polysaccharides are the phosphomannans produced by yeasts, especially species of *Hansenula*. Today phosphomannan fragments are used to characterize and isolate mammalian phosphomannosyl receptors, the peripheral lymphocyte homing receptor, and to study lysosomal enzyme targeting. Virtually all publication in these fields employ material provided by NRRC.

One of the dextran research team won the Garvan Award. In 1979 production of xanthan gum was 12 million pounds. It is now estimated that the industry has the fermentation capacity to produce 30,000 metric tons per year.

Mycotoxins

Mycotoxins are toxins produced by fungi and may be carcinogens, teratogens and mutagens. The modern mycotoxin era started when it was discovered in Great Britain that *Aspergillus flavus*, growing in peanuts, produced very toxic materials called aflatoxins. This group of toxins were very carcinogenic compounds which were heat stable and caused thousands of deaths in poultry.

Initial work in the U.S. was carried out at MIT, Food and Drug Administration, Southern Regional Research Center and at Northern Regional Research Center. Surveys conducted by the Center showed that the problem commodities were corn and milk contaminated with aflatoxin as a result of cows consuming contaminated grain. Peanuts and cottonseed were found to be sometimes contaminated with aflatoxins. Our work showed that there did not appear to be a problem in soybeans and wheat. Three types of assays were developed: one was a rapid presumptive test using the black light; the second, a more precise test used to detect aflatoxins; and the third, quantitative methods to determine the amount of toxin down to parts per billion. The black light method is universally used in monitoring corn both in the U.S. and abroad. A method of producing aflatoxin using an agitated solid-state fermentation was developed to satisfy the needs for large animal and poultry feeding studies. This fermentation process was unique in that the solid substitute is constantly shaken

Once a bin of corn is contaminated one needs to have a method for destroying aflatoxin. Work at the Center led to the development of a method using ammonia which destroys the aflatoxin. For example corn with 1,200 ppb of aflatoxin, when treated with ammonia, had its aflatoxin level reduced to 5 ppb. As far as I know, this is the only economical and safe method for destroying aflatoxin in corn on the farm. Feeding trials have shown that no toxicity develops in animals fed this detoxified corn.

Recently a fungus ecologist working at the Center has worked out the life cycle of *Aspergillus flavus* in the corn field. The fungus produces sclerotia which overwinters in the field and then sporulates in the spring; picnic beetles then carry the spores to the corn ears in the field.

Work on other mycotoxins produced by *Aspergillus* and *Fusarium* species is ongoing with the emphasis on those toxins most commonly encountered, such as zearalenone that interferes with reproduction, especially in swine. This mycotoxin work has resulted in two team awards.

Fermented Foods

Down through the years, research on utilization of soybeans has been assigned to the NRRC. Large amounts of soybeans are exported each year to the Orient where beans are used extensively for food. Soybeans, because of some undesirable characteristics, are fermented to produce healthful and pleasing foods. An exception is the production of tofu, a precipitation of soybean milk. The conditions for making different kinds of tofu were known by the tofu makers but not available to the public. The conditions required to make various kinds of tofu were studied at NRRC and the information published. Other foods such as miso, shoyu, tempeh, ontjom, and natto are all made by fermentation. USDA, as early as 1920, conducted research on soybean fermentation, especially soy sauce. Our research on miso led to explaining why U.S. soybeans were inferior to Chinese beans and the problem was overcome by using grits which brought about a more uniform colored product and a much faster fermentation. Work at the Center showed the principal yeast in shoyu and miso is heterothallic; therefore, breeding for improved strains was possible. This information was used commercially to improve the miso fermentation product. Finally, we developed a pure culture inoculum for the miso fermentation that avoided the problems associated with using old contaminated inoculum. Today this method is accepted and used extensively commercially in Japan.

A second fermented food which we studied was the little known Indonesian tempeh. This fermentation intrigued us because all the beany flavor of soybeans was destroyed and the fermentation lasted only 24 hours compared to weeks for miso. Tempeh is made by the fermentation of whole soybeans to produce a cake which is then fried or cooked. Our contributions to this fermentation were: 1) after a survey of many tempeh factories in Indonesia, we determined that only *Rhizopus oligosporus* was required; 2) a method for pure culture fermentations on an industrial scale was developed; 3) fermentation could occur in plastic containers, and this is used widely in Indonesia; 4) a method was developed to produce pure-culture dry inoculum of the mold; 5) we developed and patented fermentations of cereals and mixture of cereal and soybeans; 6) we discovered that the tempeh mold produced an antibiotic against some bacteria and, hence, explained why it is considered as a good food for people with intestinal infections; 7) demonstrated that in soybeans there is a mold inhibitor that is heat stable and highly water soluble; 8) we elucidated the presence of proteolytic and lipolytic enzymes in this soybean fermentation. Finally it should be mentioned that we made extensive studies on sufu (fermented tofu), ontjom (fermented peanut press cake) lao-chao (fermented rice or cassava) and ragi (an inoculum for various starch fermentations for food and alcohol). Besides research papers and patents we also published a book on soybean fermentation.

Fermentation in Peoria Today

Today, Peoria has 2 large fermentation companies. Archer Daniels Midland Company uses corn to make alcohol presumably for industrial uses, including as a gasoline additive. It utilizes the old Hiram Walker distillery. PMP Fermentation

Products, Inc. has rather recently opened a plant to produce sodium gluconate. Sodium gluconate is a versatile, non-toxic chemical used in cleaning and metal finishing, and has many other uses. According to their brochure they employ a continuous closed-system process which is the most advanced fermentation plant in the U.S. for making gluconate. The whole fermentation process is completely automated using a large mainframe computer which manages the entire process from the raw materials to the finished product ready to be shipped.

Honors and Awards

Many individuals and team awards have been made by the Department of Agriculture to outstanding NRRC scientists. However, Peoria area scientists have received a number of awards and honors from organizations not associated with USDA for work in the field of applied microbiology. A partial list of these awards are:

1. Three people have been elected to the American Academy of Microbiology.
2. Two scientists have been elected to the National Academy of Science.
3. Four scientists have been invited to study for one year in institutes in West Germany and England.
4. One person was elected to the Inventor's Hall of Fame.
5. One person was awarded the Third Order of the Rising Sun by the Japanese Government.
6. Three scientists were elected presidents of the Society of Industrial Microbiology, and the same three were elected presidents of the Mycological Society of America. A fourth was elected president of the U.S. Culture Collection Federation.
7. Two people have won the Pasteur Award.
8. One scientist has won the Garvan Award of the American Chemical Society.
9. The penicillin team received the Lasker Award of the American Public Health Association.
10. One person was given the Humboldt Award from Germany for a one-year study.
11. The American Institute of the History of Pharmacy and the Illinois Pharmacists Association honored the work on penicillin in 1980 by placing a special bronze plaque at NRRC honoring the work on submerged fermentation, the discovery of penicillin precursors and for finding a superior mold strain.

12. The Public Television Network Show, Nova, had considerable footage about NRRC work on penicillin in a recent program.

It also should be mentioned that there has been a constant flow of scientists and technicians from all over the world working with us and contributing to our work and they, in exchange, learning our techniques. Most of the money for this work was provided by the foreign country from which the individual came.

I believe I have now made the case that Peoria has become a Center of fermentation excellence and is known worldwide for its leadership in applied microbiology and fermentation.

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