BIOGRAPHICAL MEMOIRS

Lonnie O'Neal Ingram

December 30, 1947–June 25, 2020
Elected to the NAS, 2001

A Biographical Memoir by K. T. Shanmugam

Lonnie O'Neal Ingram was a brilliant genetic and metabolic engineer, a synthetic biologist, a microbial physiologist, and a member of the National Academy of Sciences. He pioneered the genetic engineering of bacteria as a way to produce fuels and chemicals from non-food plant materials.

Ingram was born on December 30, 1947, in Greenwood, South Carolina, and was raised in Cheraw, South Carolina, by his mother, Jean Weeks Ingram, and his adopted father, Thomas Belk Ingram, a lawyer and state magistrate. Ingram graduated from the University of South Carolina in 1969 and earned his Ph.D. in 1971 from the University of Texas. After a short postdoctoral position at Oak Ridge National Laboratory, he was hired by the University of Florida (UF) in 1972 and spent his entire academic career of forty-nine years there as a faculty member in the Department of Microbiology and Cell Science.

Ingram started his research at UF on the effect of alcohol on microbial cell membranes, and this work was further enhanced by a Career Development Award from the National Institutes of Health. About fifteen years later, his research shifted to microbial metabolic engineering, with an initial focus on ethanol production by engineered bacteria. This work expanded to further alteration of the metabolic networks of bacteria in pursuit of producing various chemicals as fuel molecules and industrial feedstock. His long-time ambition was to develop genetically engineered and metabolically adapted microorganisms that could help humankind in its efforts to overcome the environmental degradation resulting from dependence on fossil fuels.

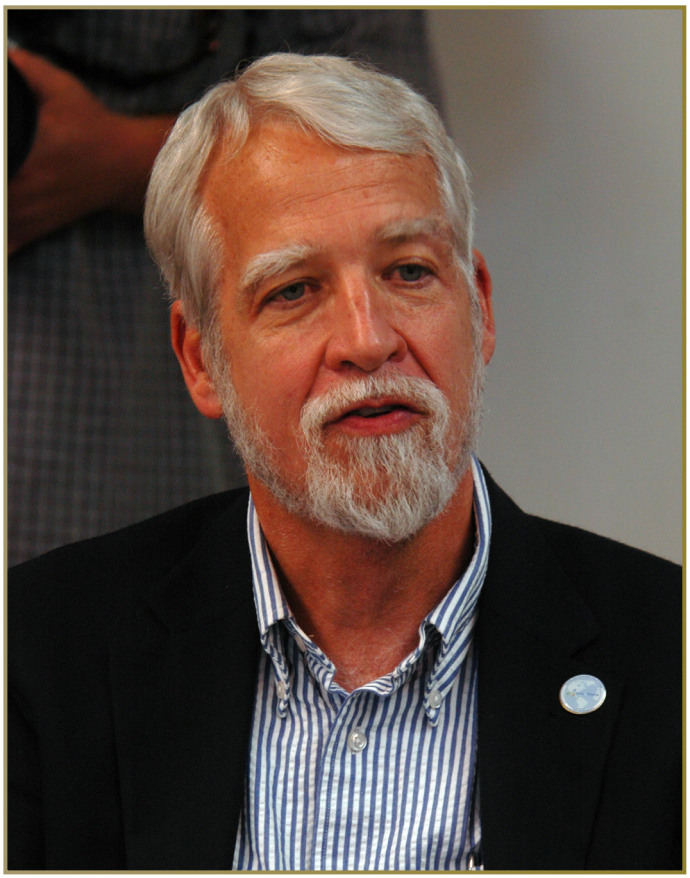

Figure 1 Lonnie O. Ingram. *Photo courtesy of University of Florida, Institute of Food and Agricultural Sciences*

Genetic changes in living systems that have led to better performance are well documented in human history. Random mutations, whether natural or induced, have produced microorganisms that are better performers in a specific environment, or produce higher product titers, among many other improvements. Other than the initial identification and selection of the microorganism with the desired trait by its phenotype, the introduction of the desired change was not directly controlled by the investigator early on in this research. The arrival of recombinant DNA technology in the

early 1970s opened the way to targeted construction of microorganisms as microbial factories for the development of desired products.

Microorganisms using their myriad complex network of metabolic pathways produce an array of chemicals that have applications in the food, health, and chemical industries. But these chemicals are either produced in small quantities or mixed with other chemicals and therefore need extensive purification. Ingram pioneered the development of genetically engineered microorganisms to produce these chemicals both in almost pure form and in large enough quantities for industrial manufacture. These innovations enabled the metabolic engineering of the bacterium *Escherichia coli* to produce ethanol as a sole fermentation product, using several naturally abundant sugars as feedstocks. It would be a pivotal point in the development of microbial biocatalysts for fuel and chemical production. His innovation was recognized by the U.S. Patent office with its award of the landmark five millionth patent in 1991.

Ingram also developed metabolically engineered derivatives of a variety of microorganisms for the production of various industrial chemical feedstocks, including D-lactate, L-lactate, succinate, acetate, pyruvate, and alanine. These innovations, well funded by federal, state, and private sources, led to more than forty U.S. and international patents. Many of these engineered microorganisms were licensed to industry for commercial chemical production.

Engineered microbial biocatalysts are only one part of the industrial fuel and chemical production processes. Equally important for cost-effective industrial processes is an efficient pathway to convert inexpensive, raw feedstock to fermentable sugars and then to the desired final product. This was Ingram's preaching point, and he developed and continuously improved these technologies. In addition to being a biologist and microbiologist, Ingram took on the role of an industrial engineer in the design and construction of a demonstration-scale biorefinery, Stan Mayfield Biorefinery, to take his processes and engineered microorganisms from lab scale to industrial scale.

Ingram was always inquisitive and dared to ask, "what if?" If he did not have the answer, he never hesitated to seek help. He was passionate about his work and could be found in his lab during evenings and weekends following up on experiments or discussing problems and solutions with colleagues, students, and postdocs. He was unpretentious, always generous and helpful to his colleagues, including this author, and collaborated with a large group of his contemporaries both in the United States and abroad without expecting anything in return. His annual Christmas parties at his home for the entire department were always a much-anticipated event. His service to the scientific community at various levels was extensive and is not detailed here for the sake of brevity.

Ingram's laboratory was always active, teeming with domestic and international groups of graduate students, postdocs, visiting professors, and scholars. Many have gone on to successful careers in academia and industry in various parts of the world, continuing Ingram's legacy. These collaborations led to some 300 refereed publications and several book chapters and reviews.

Ingram was elected to the National Academy of Sciences in 2001. His other honors include election as a fellow of the American Academy of Microbiology, the Society for Industrial Microbiology and Biotechnology, and the U.S. National Academy of Inventors, among others. He received the Distinguished Service Award from the U.S. Department of Agriculture (the agency's highest award) in 1993 and commendations from the U.S. House of Representatives and the Florida State House and Senate in 1991 for his contributions to science and renewable energy production. Ingram was an advisor to Pres. George W. Bush on cellulosic ethanol, and he was also an invited guest at the signing of the Executive Order on Biobased Products and Bioenergy. This is just a select list of the various awards Ingram received recognizing his contributions to the field of bioenergy and bio-based chemicals.

Ingram raised three children with Vicky and, after her passing, married Nancy Draffin of Charleston, South Carolina. He retired in 2017 as a distinguished professor. He was the founding director of the Florida Center for Renewable Chemicals and Fuels and served in that capacity from 1998 until his retirement. He also served as the co-director of the Florida Institute for Sustainable Energy: Energy Technology Incubator from 2007 to 2017.

He enjoyed fishing and traveling. Lonnie passed away on June 25, 2020, in Charleston, South Carolina, and is survived by his loving wife, Nancy, their children, stepchildren, and grandchildren, and his brother.

Remembrances of Lonnie Ingram

Jesus Zaldivar (*former postdoc*): "Doctor Ingram was a focused, hard-working, prolific inventor and ethical scientist. I can't say that my stay in the lab was spectacular, but labs are not supposed to be spectacular in the first place, and we're humans after all. His caring recommendation letter afterwards was no less than moving.

Over the years, I haven't forgotten three things I heard Doctor Ingram say: First, 'after you get something done, you look back and see that you could've taken a shorter path, but now it's done anyway,' which emphasized his 'go for it' approach.

The second, since he wasn't a molecular biology expert in the early days, the first thing he did was to purchase materials

and reagents and go to the lab to do it himself. That he would make mistakes didn't matter, learning how to do it was the purpose. Again, nothing is better than 'go for it' to master something.

The third, that one of his first publications ('adaptation of cell membranes,' I believe) still fresh at UF, the solo approach took so much of his time and energy, with small and slow 'return on investment.' Teamwork is faster, more effective, and allows higher throughput was the lesson. Yes, 'well done and fast,' his motto was."

Dan Li (*former colleague*): "He achieved many great accomplishments in genetic engineering of bacteria for production of fuels and chemicals from inedible plant materials. As a pioneer in this field, Professor Ingram also trained many excellent scientists all over the world. He invited me to his laboratory in 2012 and was always very kind to me. The experience of one year in his laboratory expanded my research scope."

Xuan Wang (*former postdoc*): "No doubt that I will not have my current career without his support and wonderful mentoring. He has been such an inspiring model for my career pursuits."

Jonathan Moore (*former graduate student*): "Dr. Ingram's hard work, generosity, and wisdom made a huge impact on so many people's lives. I think of him often and about how fortunate I was to have known him."

Vilas Shukla (*former postdoc*): "Prof. Ingram was one of the greatest scientist I came across during my career. By his great mentorship, he made lots of positive impacts on our lives."

Huabao Zheng (*former postdoc*): "As one of his postdocs, I keep remembering his outstanding achievements and contributions in biofuels and biochemicals research field by metabolic engineering. Especially, typical fermentation products including Alanine, lactates are being well commercialized."

Selected Bibliography

1974 With M. M. Blackwell. Macromolecular requirements for the physical initiation of cell division in a filamentous mutant of *Agmenellum quadruplicatum*. *Biochim. Biophys. Acta* 335:295–302.

1982 With L. C. Eaton. Acute effects of ethanol on biosynthesis and glycosylation of IgG1(kappa) antibody molecules in cultured P3/X63-Ag 8 myeloma cells. *Alcohol Clin. Exp. Res.* 6:459–468.

1987 With T. Conway et al. Genetic engineering of ethanol production in *Escherichia coli*. *Appl. Environ. Microbiol.* 53:2420–2425.

1991 With K. Ohta et al. Genetic improvement of *Escherichia coli* for ethanol production: Chromosomal integration of *Zymomonas mobilis* genes encoding pyruvate decarboxylase and alcohol dehydrogenase II. *Appl. Environ. Microbiol.* 57:893–900.

2002 With R. Gonzalez et al. Global gene expression differences associated with changes in glycolytic flux and growth rate in *Escherichia coli* during the fermentation of glucose and xylose. *Biotechnol. Prog*. 18:6–20.

2003 With T. B. Causey, S. Zhou, and K. T. Shanmugam. Engineering the metabolism of *Escherichia coli* W3110 for the conversion of sugar to redox-neutral and oxidized products: Homoacetate production. *Proc. Nat. Acad. Sci. U.S.A.* 100:825–832.

2006 With S. Zhou et al. Fermentation of 12% (w/v) glucose to 1.2 M lactate by *Escherichia coli* strain SZ194 using mineral salts medium. *Biotechnol. Lett.* 28:663–670.

2007 With X. Zhang et al. Production of L-alanine by metabolically engineered *Escherichia coli*. *Appl Microbiol Biotechnol*. 77:355–366.

2008 With K. Jantama et al. Combining metabolic engineering and metabolic evolution to develop nonrecombinant strains of *Escherichia coli* C that produce succinate and malate. *Biotechnol Bioeng*. 99:1140–1153.

2009 With X. Zhang et al. Metabolic evolution of energy-conserving pathways for succinate production in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* 106:20180–20185.

2015 With R. Geddes, and K. T. Shanmugam. Combining treatments to improve the fermentation of sugarcane bagasse hydrolysates by ethanologenic *Escherichia coli* LY180. *Bioresour. Technol.*, 189:15–22.

2016 With K. Gubicza et al. Techno-economic analysis of ethanol production from sugarcane bagasse using a liquefaction plus simultaneous saccharification and co-fermentation process. *Bioresour. Technol.* 208:42–48.