A Memorial Review of Jay Bailey's Contribution in Prokaryotic Metabolic Engineering

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Abstract: When mentioning *prokaryotic metabolic engineering,* most people will immediately think of Jay Bailey. Jay's contribution to this fast-growing field is evident and familiar to many. Therefore, instead of a detailed technical review, we attempt in this article to summarize his contribution and dissect reasons for his success in this area from a standpoint of one of his former students (VH) and of a colleague in the field (JCL). This short review is by no means complete and provides only a partial view of Jay's contribution to the metabolic engineering of prokaryotes. © 2002 Wiley Periodicals, Inc. *Biotechnol Bioeng* **79**: 504–508, 2002.
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RESEARCH CONTRIBUTIONS IN PROKARYOTIC METABOLIC ENGINEERING

A survey of his work points to three main areas of his research in these systems: host-plasmid interactions, metabolic engineering of central carbon pathways, and engineering of metabolic regulation. While these have been very active areas of research in the biochemical engineering community, Jay's approach was characterized by a unique combination of quantitative, systematic approaches that combined mathematical analysis and advanced analytical methods for the identification of the effects of metabolic engineering actions on the desired objective and the associated system responses. Among his many contributions, one of the longlasting values of his work lies in the way he formulated the problems and the combination of technologies for addressing these problems.

Host-Plasmid Interactions

The manipulation of cellular function through the amplification of enzyme activities has been one of the main strategies in metabolic engineering practices. This amplification is achieved by the introduction of genes of interest via a multi-copy plasmid, expecting that the increased gene copy number will eventually result in increased catalytic activity of the encoded enzymes and a correlation between gene copy number and desired phenotype can provide guidance for engineering a genetically stable strain. However, the outcome of these approaches depends on many factors: efficiency of transcription and translation, plasmid stability, and system responses to metabolic perturbation due to the presence of foreign protein and/or altered enzyme activity. Driven by the objective to quantify the correlation between gene copy number and cellular performance, Jay recognized the need for a better quantitative understanding of plasmid stability and the complex interactions between the host and the plasmid.

To quantify the plasmid heterogeneity in recombinant *E. coli* and its effects on strain performance, Jay used flow cytometry, an analytical method he first introduced in biochemical engineering, 2-D gel electrophoresis, and molecular biology methods. These studies led to a quantitative identification of effects of plasmids on cell-cycle, the redistribution of cell composition due to plasmid, and optimal process conditions, such as dilution rate in a continuous culture, for maximum plasmid and gene product concentrations.

The application of mathematical modeling to the analysis of host-plasmid interactions was one of the early demonstrations of the usefulness of mathematical modeling for the analysis of complex cellular processes for problem solving in biotechnology. Jay realized the power of the single cell *E. coli* mathematical model developed by Mike Shuler and co-workers, and with Steve Peretti they further developed this model to include plasmid related processes. Computational studies of this model provided a significant insight on the effects of plasmid on the cellular composition and allowed the quantitative investigation of interaction between components in protein synthesis machinery, such as promoter and ribosomal binding site, and cell physiology.

These mathematical studies were based on Jay's pioneer work with Sun Bok Lee on the mathematical modeling and analysis of plasmid replication and plasmid-encoded proteins . This work provided a rational, quantitative frame-

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work for the study of plasmid and the associated physiological properties, and provided the first mathematical structure for investigating the interplay between growth rate and productivity of plasmid-encoded proteins. This modeling framework used was named "genetically structured modeling" and allowed "explicit, unambiguous, quantitative mapping from nucleotide sequence to overall phenotype." In the same time period, Jay had started applying NMR for the analysis of cellular metabolism. Together with Doug Axe, they also used NMR to study the effects of plasmid on the performance of metabolic pathways.

Central Carbon Metabolism

The importance of central carbon pathways (i.e., glycolysis, pentose phosphate pathway, and tricarboxylic acid cycle) on the performance of production organisms has been recognized since the beginning of biochemical analysis. Within metabolic engineering these pathways emerged as the center of focus due to their function in providing the precursor metabolites required for the synthesis of any of the target products, such as proteins and organic acids. Moreover, the production of the required energy and redox potential by these pathways makes them even more important and introduces an additional level of complexity. Jay and his group approached this metabolic engineering challenge of central carbon pathways by employing again mathematical methods and advanced molecular biology and analytical methods.

Today, metabolic flux analysis is an indispensable quantitative tool for metabolic engineering. It was first introduced by Aiba and Papoutsakis , and Jay was one of the first to identify the power of this methodology for the study of central carbon pathways. Jay, with Ken Reardon and Thomas Scheper, were the first to apply methods for on-line culture fluorescence methods that would allow an accurate estimation of metabolic fluxes and could address the issue of underdeterminancy that stems from the fact that in every metabolic system the number of the stoichiometric constraints is smaller than the number of the unknown fluxes. Metabolic flux analysis remained an active tool within Jay's research, and in 1997, he led an interdisciplinary team that developed a novel NMR methodology for the identification of metabolic fluxes in *B. subtilis*, which has been later adapted for flux analysis in other organisms and is currently used for many industrial applications.

One of the most successful applications of metabolic engineering from Jay's group has been the expression of *Vitroescilla* hemoglobin (Vhb) in *E. coli*. Chaitan Koshla and Jay hypothesized that expression in *E. coli* of a hemoglobin-like molecule which has been found to be induced in the obligatory aerobic bacterium *Vitroescilla* under oxygen limitation, could improve strain performance under oxygen limitation. They isolated the gene from *Vitroescilla* and when they expressed it in *E. coli* they indeed observed increased rates of oxygen consumption, faster growth, and higher final cell densities. This result led to a number of studies that showed that Vhb expression in many different hosts could significantly enhance strain performance, ranging from protein synthesis in *E. coli* to antibiotics production in *Streptomyces*. The exact mechanism of action of Vhb, that was responsible for these results, was not clear, and from 1988 until his last days, Jay worked with a number of talented postdocs and graduate students towards the dissection of the regulatory and physiological mechanisms of Vhb function. All of these approaches involved the employment of advanced molecular biology and analytical methods, and mathematical modeling and analysis.

The metabolic engineering of the central carbon pathways has been one of the active areas of research in biochemical engineering and Jay's group has been involved during the last 25 years. Some examples of his work involve the manipulation of key enzymes in central carbon pathways of *E. coli* for improving ethanol production, decreasing acetate production, and overcoming the precursor metabolite and energy limitations of amino acid production. While all of these studies provided important insights into the central carbon pathways, their real value lies on teaching how advanced analytical techniques, such as in vivo NMR, and mathematical frameworks, such as flux analysis and kinetic modeling, can be combined to address problems in metabolic engineering. One of the best examples is the work by Chen et al. where metabolic flux analysis and kinetic modeling provided the framework for integrating information from in vivo NMR and fermentation performance and allowed the explanation of metabolic responses that otherwise would be very difficult to interpret.

Engineering of Metabolic Regulation

As a process and control engineer, Jay identified very early the role of metabolic regulation as an obstacle in metabolic engineering applications and as a potential target for metabolic engineering. His initial approach to this problem was based on mathematical modeling and analysis. The work with Sun Bok Lee (see above) has been one of the first approaches in understanding genetic regulation in the context of recombinant DNA technology. One of the most successful applications of this modeling approach, and of mathematical modeling in general, is Jay's work with Wilfred Chen and Sun Bok Lee. They used genetically structured models to evaluate the effectiveness of alternative molecular designs of expression systems. Based on these modeling studies, Wilfred and Pauli Kallio constructed and characterized an experimental realization of the predicted optimal design and the results were in "embarrassing agreement with the model predictions" (as Jay described it in one of his presentations). The concepts of this work were also used for the design and experimental implementation of "metabolic switches" that enable the controlled switch of metabolic flow from one pathway to another.

The tight control of metabolic pathways through allosteric regulation of enzyme activity had been long identified as a barrier in efficient manipulation of metabolic fluxes by

overexpressing enzymes alone. Jay worked with one of us, and in collaboration with Chris Floudas, on the development of a mathematical and computational framework for the analysis and design of regulatory architectures around metabolic pathways. The methodologies employed advanced optimization methods and concepts from process system engineering. Application of these frameworks provided guidance for experimental approaches in improving ethanol production by *E. coli*, and Jay's work with Uwe Sauer is one of the few examples of metabolic engineering of central carbon pathways through engineering of enzyme regulation.

JAY BAILEY'S SUCCESS

Going through Jay's papers, two questions arose: What made Jay successful, and what can we learn from Jay's success? These are open-ended questions to which no definite answers exist. From a personal standpoint, we attempted to analyze his success history, hoping to provide some insight to the above questions and provoke thinking in these areas.

One important element for the success of an academic researcher is the research philosophy, which is the guiding principle for selecting problems to work on. In most cases, research problems are strongly influenced, if not dictated, by research inertia and funding opportunities. Many continue to work on the same problem with small changes in directions until funding becomes scarce. Jay did not follow this pattern. His vision guided his research directions before external pressure became severe. He cleverly capitalized on his expertise in every turn of research to provide continuity, and yet did not let inertia dominate his course of research. In a way, he skillfully practiced the most successful tactics in both evolution and rational design. In evolutionary terms, we see both "vertical evolution" and "horizontal transfer" in his research history. On the other hand, we also see clearly his effort in "rational design" of research courses.

Vertical Evolution

Jay was trained as a mathematical modeler in reaction engineering. His work started from mathematical modeling of catalysis and chemical reaction engineering. Relatively early in his career, he made a rather sharp turn to the biological systems, where he began by applying mathematical modeling techniques to bioprocess-related problems at the macroscopic level. Soon he recognized that engineers needed to enter the cell and deal with biological details. He was among the first to appreciate the biological intricacies in bioprocessing and decided to face the challenges directly. Again, his first foray into the cell was mathematical. As discussed above, his modeling of plasmid replication set the foundation of much of his later work. He continued this mathematical work by applying modeling analysis to gene expression, metabolic fluxes, and cell cycles. In just about every problem he engaged, one can find the impact of mathematical analysis. This emphasis on mathematical analysis of biological systems at times was viewed as an academic practice, but Jay's persistence in this central theme and his focus on clear definition of problems eventually prevailed. He recognized that mathematical models were not embraced by life scientists, and thus insisted on theoretical soundness and biological relevance in modeling. He stated in one of his articles, "Therefore, mathematical modeling does not make sense without defining, before making the model, what its use is and what problem it is intended to help to solve." And he repeatedly cautioned against "overselling" models.

Now the importance of mathematical modeling in biological systems is appreciated much more and the term "systems biology" has been widely used in the scientific community. During his fight against cancer, he told one of the authors that his research was to focus more on mathematical analysis of biological systems, as he saw a clear niche and need in this area.

Horizontal Transfer

At the same time, Jay did not confine himself to tools at hand. He actively sought out for new and powerful research methods. He acquired expertise in physical methods such as EPR, flow cytometry, and NMR, and biochemical tools such gene cloning. These tools, although widely adopted in scientific communities, were not commonly applied to bioprocessing problems. Jay's applications to engineering problems were novel, unique, and visionary. Flow cytometry becomes one of the most powerful tools for screening of novel proteins; NMR has attracted new attention for metabolomic applications; and cloning is now commonplace in engineering biotechnology. In every project, Jay attempted to use cutting-edge technology to solve problems. He stated in one of his lectures "unless we make a conscious effort to learn new tools and methods, to understand concepts from presently unfamiliar areas, and then to integrate these with creatively chosen new problems in our field, we risk stagnation and irrelevance." (Bailey, 1998) These evolutionary events in Jay's research were often driven by industrial needs. He made special efforts to work with industry, identify problems important to industry, and then suggest solutions based on sophisticated reasoning and cutting-edge technology.

Rational Design

Analyzing Jay's research using evolutionary terms risk an erroneous impression that Jay's research was drifting by external pressure. On the contrary, we see that he made a significant effort to chart the course of not only his research, but also the field as a whole. The most prominent example is his 1991 *Science* article "Toward a Science of Metabolic Engineering," in which he articulated the essence of Metabolic Engineering and pointed to some promising examples. Many of the examples cited in that article later became highly important projects in industry. Jay, along with others,

recognized the emergence of Metabolic Engineering as a cohesive field, distinct from Genetic Engineering. He articulated the need for a new identity and pointed out the promising future. The findings presented in this article catalyzed the development of this field, which has now grown to include not just the engineering of metabolic pathways in the strictest sense, but also the alteration of cell function in any definable manner.

Jay also recognized that the field of Metabolic Engineering was still largely defined by a collection of examples. The need for an underlying fundamental theory is evident. He repeatedly called for the theoretical development of Metabolic Engineering and close coupling between experimental and modeling efforts. This was reflected in his last attempt to chart the direction of Metabolic Engineering in the form of a conference. Unfortunately, he did not live to see through it.

CLOSING REMARK

Jay's work was characterized by quantification through state-of-the-art technologies and analysis of information through mathematical modeling and computational analysis. His efforts to incorporate new experimental tools allowed him to stay cutting-edge and relevant. His persistence in mathematical analysis provided a rational basis in this fast evolving field. Jay undoubtedly was one of the most successful engineers who advocated for direct engagement of biology. It is now widely appreciated that understanding the fundamental principles, not just in physics and chemistry, but also in the details of biology, can greatly advance process development. Finally, Jay's keen articulation of his vision as well as the field's sentiment made him a great teacher. His contribution to the field of Metabolic Engineering will be long remembered.

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