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Computers and Chemical Engineering 29 (2005) 509–517
Computers & Chemical Engineering
www.elsevier.com/locate/compchemeng

Antibiotic resistance as a model for strain engineering

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Received 22 September 2003; received in revised form 3 February 2004
Available online 30 November 2004

Abstract

Metabolic engineering has been defined as "the improvement of cellular activities by manipulation of enzymatic, transport, and regulatory functions of the cell with the use of recombinant DNA technology" [Bailey, J. E. (1991). Toward a science of metabolic engineering. *Science, 252*, 1668–1675]. As such, it mimics the processes of natural evolution by engineering new traits through directed alterations in gene expression, mutation, disruption, or copy number. Therefore, studies of the mechanisms by which new traits evolve in nature hold important lessons that can be used to guide the design of novel metabolic engineering strategies. Likewise, lessons derived from laboratory-based metabolic engineering studies might enable the development of improved methods for better controlling the evolution of new traits in nature. In this article we will discuss these concepts within the context of an important and alarming example of natural evolutionary forces, antibiotic resistant bacteria. We will describe several examples of the major mechanisms by which antibiotic resistance has evolved in nature and relate these to previous, current, and possible future metabolic engineering efforts.

Keywords: Antibiotic resistance; Metabolic engineering; Systems biology

1. Introduction

The intellectual framework of metabolic engineering is built upon the integration of biological information in an attempt to induce higher order principles that govern cell behavior. As such, metabolic engineering and the emerging field of systems biology share an over-arching emphasis on revealing general biological principles from the analysis of the regulation and activity of biological networks ranging from gene sequence to gene expression to metabolic flux (Stephanopoulos & Gill, 2000). The engineering of new traits or re-engineering of existing traits, which is dependent upon such biological networks, is a major thrust of metabolic engineering. Specifically, metabolic engineering involves the modification of the genetic makeup of an organism in an attempt to re-direct cell behavior in a specific manner (Bailey, 1991). This might involve, for example, engineering increased or decreased expression of a gene that is thought to influence production of a valuable chemical product. While such an example may appear to be straightforward, it is complicated by the fact that metabolism forms a network of chemical reactions that are mutually interdependent and that incommensurately influence overall network activity, which itself influences the relative fitness of an organism in a particular environment. Therefore, any attempt to engineer flux through a specific pathway in an organism can result in secondary effects that may include a reduction in the overall fitness of the organism and, as a result, reduce the attractiveness of the engineering strategy (Bailey, 1999). Antibiotic resistance provides numerous examples of how nature has approached this problem. In particular, antibiotic resistance exemplifies how bacteria routinely develop new phenotypes through combinations of creative and hard to predict mechanisms and the importance that environment plays in selection and maintenance of such phenotypes. As such, it serves as a model to elucidate underlying evolutionary mechanisms that might be applied to the development of future metabolic engineering efforts.

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doi:10.1016/j.compchemeng.2004.08.028

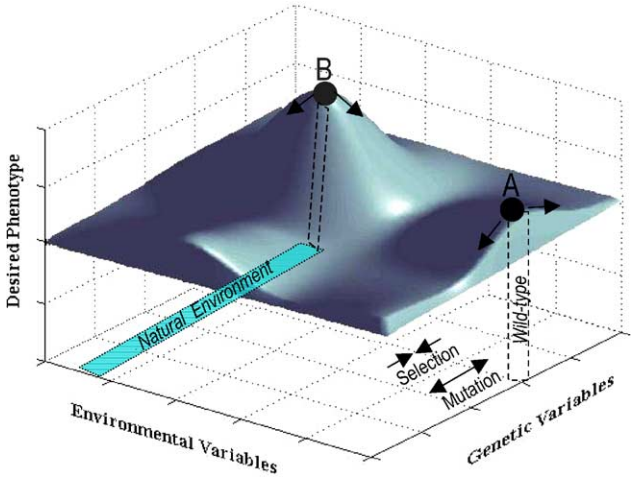

Fig. 1. An example fitness landscape. Point A represents the wild-type genotype and point B represents a phenotypic optima expressed only in the native environment.

1.1. The fitness landscape

The concept of the fitness landscape provides a useful way to visualize the evolution of new traits by plotting phenotype as a function of genotype and environment (see Fig. 1) (Kauffman, 1994; Wright, 1932). The phenotype of an organism corresponds to an observed or measured trait. The genotype of an organism is the collection of genetic information that defines that organism (i.e. the sequence of the genome) and forms the basis of associated phenotypes. Genetic mutations, either random or engineered, alter the genotype of an organism, which can be represented as movement along the genetic axis in Fig. 1 to a new location corresponding to a new phenotype. The challenge in industrial strain engineering programs is to engineer the traversal of the fitness landscape of a particular species, through either genetic or environmental alterations, to a location at which an economically viable process can be operated. A closely analogous challenge exists in clinical microbiology. Physicians employ antibiotic treatments to engineer the host environment such that the fitness of the pathogen of concern is sufficiently reduced. A key concept in both of these examples is that mutation and selection act to expand and contract, respectively, the genetic diversity of the relevant population until only the genotypes most fit to a particular environment remain (points A and B in Fig. 1). When considering cell populations in this manner, several key questions come to mind: What are the costs and benefits associated with each of several different mechanisms capable of conferring a new trait to a relevant organism? More specifically, are there environments where any genetic alteration away from the wild-type will result in decreased fitness (i.e. movement away from point A along genetic axis)? If so, metabolic engineering efforts should first focus on identifying an economically viable process environment followed by genetic alterations to further improve expression of the relevant phenotype as needed. Similarly, are there genotypes for which the maximum fitness value is found only in the natural environment of the species (point B in Fig. 1)? If so, metabolic engineering efforts might initiate by identifying the genetic basis of the phenotype in the natural host and then transferring the trait to a host organism better suited for the intended application. Variations on these themes can become considerably more complicated. For instance, what secondary alterations are capable of compensating for mutations that alter a phenotype in one environment but have a large fitness cost in a different environment? These questions are all of fundamental importance to not only engineering industrially useful strains but also to treating infectious diseases. In industrial strain engineering programs it is common to find strains that have a high product yield but are restricted to growth in environments that are not economically viable at the industrial scale (Ohnishi, Mitsuhashi, et al., 2002). Analogously, antibiotic resistant mutants have a high fitness value in the presence of the antibiotic but often suffer from a reduced fitness state compared to the wild-type strain when grown in the absence of the antibiotic (Bjorkman et al., 1998; Sander, Springer, et al., 2002; Schrag & Perrot, 1996).

In this review, we will expand upon these issues by providing examples of the mechanisms by which bacteria evolve to resist the effects of different antibiotics and the relative costs and benefits of such evolutionary changes to the bacteria as environmental conditions are altered. We will discuss specific aspects of resistance mechanisms that are most relevant to metabolic engineering and that provide some lessons for future metabolic engineering efforts. In particular, we will describe enzyme-based resistance mechanisms that often have a low fitness cost to benefit ratio, permeability-based resistance mechanisms that arise through the pleiotrophic effects of altered regulatory systems, efflux pump mechanisms that might be used to engineer tolerance to toxic components of relevant environments, and antibiotic target mutation-based mechanisms that often have a high fitness cost to benefit ratio. It is our hope that this review will encourage metabolic engineers and systems biologists to bring their skills to bear upon further elucidating the design principles underlying this and other examples of natural evolution.

2. Antibiotic resistance mechanisms

Antibiotic resistance accounts for close to US$ 30 billion/year in increased health care costs and is now a factor in almost all nosocomial infections (NIH, 2000). Antibiotic resistance emerges very rapidly after the introduction of an antibiotic and now exists to some extent for all antibiotics in clinical use (CDC, 2000; Shnayerson & Plotkin, 2002). Clinically useful antibiotics fall into a number of major classes that target critical processes required for bacterial replication and/or survival. Several of these classes, along with their structures, targets, and known mechanisms of resistance, are summarized in Table 1. Most of the known