Commentary

Chassis organism from *Corynebacterium glutamicum*: The way towards biotechnological domestication of Corynebacteria

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For a long time, Corynebacteria have been the organisms of choice for industrial bioproduction of amino acids. Later on, Corynebacteria have also been used for making biofuels and a suite of added-value chemicals. In this issue of *Biotechnology Journal,* Unthan et al. [1] provide a splendid example of how systems and synthetic biology approaches are instrumental for significantly contributing to the value of *Corynebacterium glutamicum* as a platform strain for industrial applications. To this end, genomic segments that appeared to be non-essential for maintaining every desirable trait in *C. glutamicum* were delineated. A massive, recursive deletion of each (or most) of such apparently useless DNA was then carried out to verify the relevance of the excised sequences, and the resulting *C. glutamicum* variants were tested under various growth conditions [1]. This approach produced not only interesting new strains but also raised new questions on how to design reliable microbial chassis that fulfil the biotechnological promise of synthetic biology.

Current efforts to genetically redesign microorganisms are reminiscent of the long tradition in domesticating animals for human benefit [2]. In both cases, the candidates should be pre-endowed with a number of qualities that make them promising for later exploitation. Then, non-desirable traits must be eliminated and desired traits nurtured. Unfortunately, as is the case with animals, not all attractive bacteria are amenable to such a biotechnological taming. Out of the many thousands of species known in the size range 20–2000 cm, we have been able to domesticate less than 50 types. Some animals,

attractive as they are, seem to be completely recalcitrant to domestication despite numerous attempts (e.g. the zebra horse). Perhaps the same holds true for microorganisms: the variety and activities of them are immense, but only a few are pre-endowed with the key requirements that makes these microorganisms amenable to a serious domestication effort. The list of species that have made it to mainstream industrial biotechnology is quite short and it is likely not to expand much in the near future. Availability of suitable molecular tools for genome editing is a necessary condition, but not sufficient, as the robustness and metabolic vigour that are required in industrial operations are often missing in typical platform strains (e.g. *Escherichia coli*) or model species (e. g. *Mycoplasma*). But the explosion of many methods for bacterial genome editing [3, 4] allows a serious reshaping of genomes to keep and enhance the best of the properties of a production strain while removing undesirable ones.

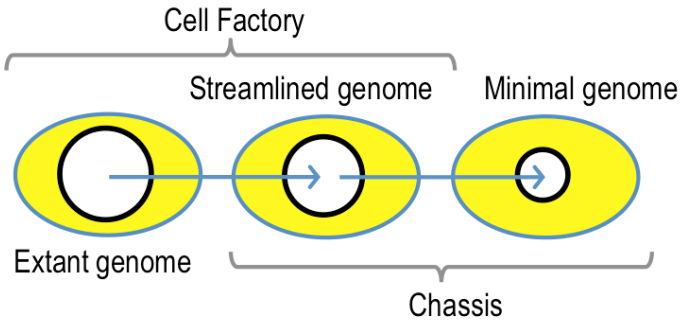

Figure 1. Genome reduction in biotechnology. Naturally occurring bacteria can already be used for industrial applications or their genomes can be rationally streamlined (e.g. non-necessary or detrimental functions deleted) for improving their properties as cell factories. Genome reduction can go further by keeping only the minimal functions necessary for sustained life. As discussed by Unthan et al. [1] such an extreme minimization might be counterproductive rather than beneficial in bacteria destined for biotechnological applications. Instead, rational removal of a limited number of genomic segments should produce robust chassis able to endure the actual operating conditions of production processes.

Two agendas stumble across each other in synthetic biology. On one hand, efforts are directed to identify the least number of genes that sustain life and metabolic functions of a given species i.e. what one could call “the quest for the minimal genome”. Creating a cell with a minimal genome is obviously a fundamental research endeavour directed to understand the origin of life and the basic framework of living systems. In addition, as shown in the studies on genomes of endosymbionts [5], the loss of compositional complexity is associated with a gain in relational complexity and environmental dependence. In this sense, it is uncertain that genomes with extremely reduced genetic information will be more predictable and easier to engineer, as claimed by some synthetic biologists. Minimal genomes may in fact not to be useful at all for biotechnological applications, at least in the near future.

On the other hand of the synthetic biology agenda is the more mundane deep genome editing aimed at removing limitations or flaws within bacterial species in industrial settings — a sort of "remove what bothers and leave the rest" approach. This approach has been very useful thus far in *E. coli, Bacillus* and *Pseudomonas putida* [6]. Now, as shown by Unthan et al. [1] the time is ripe for a similar endeavour in Corynebacteria. Although some members of this Gram-positive bacterial genus are notorious for their pathanogenicity, others have been for a long time stars of the industrial production of amino acids [7] and more recently, of a plethora of other added value products and biomolecules [8, 9]. The objective of this paper in *Biotechnology Journal* by Unthan et al. [1] was not to achieve a minimal genome, but to develop a reliable platform strain of *C. glutamicum*; a chassis, if one likes the synthetic biology jargon, that is emancipated of every identifiable hurdle left behind by its natural and evolutionary history (Fig. 1). Such useless segments make > 20% of the genome of *C. glutamicum*. Many of such deletions enable this bacterium to perform much better under virtually all conditions tested, however, some deletions were unexpectedly detrimental. Perhaps there is a level of cellular organization encrypted in the genome that becomes offset when the relative position of the targeted DNA sequences are accidentally altered [10]. This may affect also the outcome of combining different deletions in the same cell. While this opens a suite of intriguing questions that will need attention in the future, the work of Unthan et al. [1] will remain a landmark of the biology and biotechnology of Corynebacteria.

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