From dirt to industrial applications: *Pseudomonas putida* as a Synthetic Biology chassis for hosting harsh biochemical reactions

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The soil bacterium *Pseudomonas putida* is endowed with a central carbon metabolic network capable of fulfilling high demands of reducing power. This situation arises from a unique metabolic architecture that encompasses the partial recycling of triose phosphates to hexose phosphates — the so-called EDEMP cycle. In this article, the value of *P. putida* as a bacterial chassis of choice for contemporary, industrially-oriented metabolic engineering is addressed. The biochemical properties that make this bacterium adequate for hosting biotransformations involving redox reactions as well as toxic compounds and intermediates are discussed. Finally, novel developments and open questions in the continuous quest for an optimal microbial cell factory are presented at the light of current and future needs in the area of biocatalysis.

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**Introduction**

Metabolic engineering seeks the use of our current knowledge about live systems for designing and creating new biological agents that can function as biofactories of value-added products [1,2]. In recent years, Synthetic Biology (SynBio) has supported this agenda by providing a wealth of tools and strategies for the rational design of predictable DNA circuits and biochemical devices [3]. One focus of this ambitious program is the design of genetic constructs containing the information necessary to synthesize the product(s) of interest from the corresponding substrate(s). Yet, such DNA constructs must deploy their encoded properties into suitable cells that act both as physical containers and as providers of the molecular machinery necessary for heterologous gene expression. This objective leads to the important concept of chassis [4], that is, live platforms able to host and sustain the molecular transactions necessary for expressing the information encoded in purposeful DNA designs.

The number of SynBio-related technological advances increased greatly over the years, enabling metabolic engineers to design and obtain robust biocatalysts for targeted production of different types of biomolecules [5,6–8]. However, relatively less attention has been paid to selecting optimal microbial cells in which these biotransformations can be implanted — in particular for providing the right biochemical frame to obtain the desired product(s). Note that, as elegant and efficient the design of a SynBio genetic circuit may look on paper, only the background biochemical network and metabolic connectivity make its adequate operation possible. The importance of selecting new robust bacterial chassis is therefore a must to expand the number and scope of potentially useful biocatalysis and metabolic engineering endeavors. In contrast with this overarching purpose, we currently rely on a comparatively limited number of microorganisms to perform a continuously increasing number of practical applications.

In this context, the relevant question is about the features required in an ideal, bacterial-based SynBio chassis. Alas, the physical and spatial shape of a bacterial cell, its genomic complement, and its default gene expression machinery are usually not enough to meet the challenge. An optimal chassis should also provide a distinct metabolic and biochemical milieu for fueling whatever the property of interest has been genetically implanted in it, an scenario that cannot be taken for granted a priori.

**Select what you need: essential and non-essential functions in a SynBio chassis**

Since ideal SynBio constructs should operate autonomously in respect to the whole cell it is desirable that the bacterial chassis holds the minimal information needed [1] to fulfill the basic biological functions of growth and
maintenance and [ii] to run the necessary machinery to curb adverse conditions that can appear during biocatalysis. In other words, genes encoding non-essential genetic information for survival could be removed from the bacterial genome [9], which leads to the notion of **minimal bacterial genome** (Box 1)—and also to the design of such genome **de novo** [10**]. It should be noted that the idea of basic biological function is dependent on the context in which it is discussed. Recent work on quantitative biology of *Escherichia coli* has shed new light on the complex interactions between DNA replication, transcription, and RNA translation [11**], but the essentiality and relevance of which is sometimes difficult to ascertain a priori. Also note that a genome is not simply a collection of relevant genes: an organization of the genes with respect to one another is needed [12–14]. In bacterial chromosomes, in which there is a single origin of replication (thereby marking the beginning of the replication), the terminus of the chromosome is usually located near half of the entire genome DNA [15,16]. Also, there is a preferred orientation of the important genes with respect to the orientation of the replication fork movement and the 3D architecture of the bacterial chromosome [17,18]. In other words, the construction of a minimal genomic **chassis** is not just a matter of trimming DNA off, but also of carefully selecting the regions in the chromosome in which such engineering is feasible. A bacterium with such minimal genome would be not only more predictable and easily controllable [19], but it should also display reduced interactions between the cellular machinery and the genetic construct, which could otherwise lead to the appearance of unexpected reactions or phenotypes [20,21]. The microorganism of choice should be also amenable to targeted genetic modifications, with low spontaneous genetic variability, and should be equipped with a robust metabolism to accommodate the bio-reactions encoded in the genetic graft in time and space. Due to the current technological limitations to create completely artificial cells [22], a preferred microbial **chassis** must be obtained from bacterial species that have been domesticated and/or modified to work in the laboratory and the industry as well.

A suite of microbial hosts have been considered for metabolic engineering endeavors; with *E. coli*, *Bacillus*, *Corynebacterium*, *Streptomyces*, and yeast as the most widespread platforms [23–27]. Cyanobacteria are also useful for light-fueled and CO₂-fueled industrial bioprocesses [28]. The selection of a host obviously depends on the specific application intended. Alas, no single naturally-isolated bacterial strain possesses all the desired characteristics of the ideal **SynBio chassis** (Figure 1). Soil bacteria, however, could satisfy many of these requirements because of the conditions they naturally face in niches in which they thrive, particularly in terms of stressful physicochemical surroundings.

### Box 1 A glossary of Synthetic Biology and metabolic engineering-related jargon relevant to the design of microbial cell factories.

| **Chassis** | A live platform in which the user plugs-in and plugs-out heterologous genetic constructs. The term is taken from car engineering and it evokes the internal framework supporting a man-made object. **Chassis** embodies at least four different aspects: [i] the physical container of the genetic constructs, [ii] The genomic skeleton and the cellular machinery necessary for heterologous gene expression, [iii] the biochemical network in which the activities encoded by the gene implant are nested, and [iv] the spatial scaffold for the genetic and metabolic graft to take place in space and time. |
| **Orthogonality** | This concept entails the autonomy of any implanted component (e.g., genetic or metabolic) with respect to the extant cell genetics and physiology. **Orthogonality** is a requisite for programmability, a desirable trait in any microbial cell factory in which the implanted components can be externally controlled in a context-independent fashion. |
| **Minimal genome** | The genetic complement of a cell in which the individual components are modified such as the only remaining functions are those strictly necessary for replication and maintenance (and probably resistance to stress). Note that the elements needed for these functions also depend on environmental conditions. |
| **EDEMP cycle** | A novel metabolic architecture of *Pseudomonas putida* encompassing activities belonging to the Entner–Dudoroff, the Embden–Meyerhof–Parnas, and the pentose phosphate pathways. The operation of this cycle enables the bacterium to recycle part of the carbon source (from trioses phosphate to hexoses phosphate) and to adjust NADPH formation. |
| **Synthetic morphology** | The targeted modification of the cell shape or social behavior in response to the implantation of a dedicated genetic circuit. |

**Pseudomonas putida** as the starting point for a robust physical and metabolic **SynBio chassis**

Bacteria are endowed with versatile metabolic lifestyles that allow them to adapt to changing conditions, some of them rather adverse (e.g., oxidative stress, temperature challenges, and osmotic perturbations). Such adaptability is intrinsically encoded in their metabolism: environmental bacteria isolated from contaminated soils, for instance, display [i] a broad collection of transporters and enzymes to catabolize many different (and structurally unrelated) carbon sources and [ii] a set of regulators that provide the flexibility to quickly adjust to different conditions. In the case of Pseudomonads, for instance, all the metabolic machinery is geared to generate the biomass precursors, energy, and reducing power necessary to ensure the survival of the organism in virtually any hostile conditions.
Desirable characteristics in an ideal physical and metabolic Synthetic Biology chassis based on Pseudomonas. The figure sketches the journey of a Pseudomonas isolate from soil up to industrial production and summarizes the main traits needed in a chassis at the different levels of cellular organization: genomic, metabolic, and physiological. No naturally-occurring microorganism conforms with all the requirements in such ideal chassis, and Synthetic Biology and rational metabolic engineering help fulfilling these requirements. Note the cyclic nature of the manipulations needed to reach such objective.

environmental niche [29]. A particular example within this clade is the soil bacterium Pseudomonas putida.

P. putida is an ubiquitous rhizosphere colonizer that belongs to the wide (if somewhat fuzzy) group of fluorescent Pseudomonas. P. putida KT2440 is the best-characterized saprophytic laboratory Pseudomonad, that has retained its ability to survive and thrive in the environment [30], for which the entire chromosome sequence is available since 2002 [31], and this genomic data has been recently revisited, further multiplying our knowledge about this bacterium [32]. This strain is a well-established host for cloning and gene expression, displays solvent tolerance [33], and is endowed a remarkable capability to degrade aromatic compounds, such as toluene or m-xylene [34], so it is considered a model organism for biodegradation studies and a production platform [35,36]. Also, P. putida presents a high tolerance to oxidative stress [37] that is of interest for several technical applications, such as biofuels production [38]. Such properties, together with a plethora of SynBio tools tailored for targeted genetic manipulations [39–42] and the availability of four genome-scale constraint-based metabolic models for in silico studies [32,43–45], position P. putida as one of the preferred contemporary SynBio chassis [46]. Several industrial processes based on P. putida are currently being exploited for the production of fine chemicals (e.g., 2-quinoxalinecarboxylic acid, 5-methylpirazine-2-carboxylic acid, chiral amines, and 4-[6-hydroxyypyridin-3-yl]-4-oxobutyrate) [35], and rhamnolipids, terpenoids, non-ribosomal peptides, and polyketides are also in the industrial pipeline at different degrees of development [36]. P. putida has recently been proposed as an useful cell factory for the valorization of lignin [47], highly cross-linked phenolic polymers derived from vegetal biomass and a very difficult-to-degrade substrate [48], a situation which qualifies as a harsh biotransformation as it has to be executed in the presence of a plethora of inhibitory compounds and highly reactive intermediates. The intrinsic heterogeneity of this polymer leads to complex mixtures of aromatic compounds after pre-treatment,
depolymerization, and hydrolysis of lignin. *P. putida* strains have been also engineered to use these aromatic compounds as the substrate to produce biopolymers [48], *cis,cis*-muconate and adipic acid [49], and commodity chemicals lactate and pyruvate [50]. Recent advances in targeted evolution of artificial enzymes [51], use of xeno-nucleic acids [52], and genome re-coding [53,54] will certainly pave the way for new and exciting developments. In the meantime, the broad applicability of this soil bacterium in sophisticated industrial bioprocesses (and, in general, the overall physiology of *P. putida*) stem from a set of unique biochemical capabilities, not only by the abundance of peripheral pathways for aromatic carbon substrate degradation but also in the very central routes involved in carbon metabolism.

**The atypical central carbon metabolism of *Pseudomonas putida***

Building on the metaphor of microbial cells as computers making computers [55], one could identify a software (i.e., the recursive transfer and trapping of information) and a hardware (i.e., the physical machine that executes the software). In a bacterial cell, the dynamic coupling between these two main components is mediated by chemical interchange brought about by metabolism [56,57]. Yet, what is the *bona fide* definition of central carbon metabolism? This seemingly simple question accepts rather flexible answers that are dynamically changing over time — sometimes even differing from classical definitions found in biochemistry textbooks. The prevailing vision lists a number of metabolic pathways as the core carbon metabolism, such as those found in yeast or *E. coli*, including the Embden–Meyerhof–Parnas (EMP) pathway, the pentose phosphate (PP) pathway, the Entner–Doudoroff (ED) pathway, and the tricarboxylic acid (TCA) cycle. These routes can be used in very different ways (sometimes interconnected) depending on the organism considered, the prevailing environmental conditions, and the carbon source catalyzed [58–60]. Using *P. putida* KT2440 as a model bacterium, Sudarsan et al. [61] challenged this classic meaning of central metabolism, indicating that this definition may be equivocal without proper experimental validation, since microorganisms adapt their metabolic machinery according to specific environmental conditions. Regardless of the carbon source and the metabolic steps used for its breakdown, central carbon metabolism includes the metabolic pathways necessary for the synthesis of the 12 known essential biomass precursors (Box 2).

Within this framework, the genome of *P. putida* KT2440 contains all the genes encoding enzymes that make the three prominent catabolic pathways for sugars in bacteria: the ED pathway, the EMP pathway, and the PP pathway. A notable exception is the glycolytic enzyme 6-phospho-fructo-1-kinase, Pfk, that catalyzes the transformation of fructose-6-P to fructose-1,6-bisphosphate [62]. The absence of Pfk in *P. putida* immediately rules out the possibility of glucose degradation through the EMP pathway (a feature shared with few other microorganisms apart of *Pseudomonas* species, such as *Shewanella oneidensis* [63]). Glucose is transformed into 6-phosphogluconate through a series of convergent pathways [64], and this metabolite is processed almost exclusively (96%) through the Edd and Eda enzymes (i.e., the ED pathway) with only a very small fraction of about 4% entering the PP pathway. Edd transforms 6-phosphogluconate to 2-keto-3-deoxy-6-phosphogluconate, and then this intermediate is split by Eda into pyruvate and glyceraldehyde-3-P. ED metabolism is far more widely distributed in Nature than previously thought [65], the enzymes being highly conserved among bacterial and even archaeal species. Microorganisms as *Rhodobacter capsulatus* and *E. coli* run a linear ED pathway, only induced when the cells grow on particular carbon sources, and the prevalent idea based on many studies with Enterobacteria was that the ED pathway is a rather ‘accessory’ route for sugar degradation [66]. Interestingly, *P. putida* is also equipped with a dedicated ED pathway for sulfoquinovose [67], a prominent source of organosulfur in Nature.

By adopting a multi-omic approach, glycolysis has been recently demonstrated to operate cyclically in *P. putida* [68**]. The set of reactions which comprise the so-called EDEMP cycle (Box 1) includes the ED route, part of the EMP pathway (operating in a gluconeogenic mode, in the same way as when glycerol is used as the substrate [69,70]), and elements of the PP pathway. Specifically, part of the pool of triose phosphates is recycled by the EDEMP cycle back to glucose-6-P (Figure 2). Under balance growth conditions with glucose as the only carbon source, the EDEMP cycle mediates a recycling of ca. 10% of the triose phosphates generated by the ED pathway. The rest of the carbon skeletons is metabolized into acetyl-coenzyme A to produce biomass precursors and reducing equivalents through the TCA cycle. Growth experiments with *P. putida* mutants deficient in EDEMP cycle enzymes indicated that this operation is essential. Yet, apart from the explicit recycling of carbon structures (and the generation of intermediates such as fructose-1,6-bisphosphate, which could not be obtained otherwise [71]), the operation of a cyclic glycolysis has important consequences in the overall redox balance of *P. putida*, which has been shown to be quite robust [72**]. The core glycolytic metabolism is nested in a larger biochemical network that comprises, among other features, the tightly-regulated formation and hydrolysis of polyhydroxalkanoates [73]. This unique metabolic architecture strengthens the biochemical pillar of the ideal chassis in which SynBio grafts are to be implanted (see Box 1).

**Metabolism, resistance to oxidative stress, and bacterial lifestyle**

The EDEMP cycle of *P. putida* plays a role in adjusting NADPH synthesis to sustain anabolic demands and to
Box 2 The 12 precursor metabolites of central carbon metabolism in bacteria.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Metabolic pathway from which it derives</th>
<th>Building block(s) obtained from it</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-6-P</td>
<td>Embden-Meyerhof-Parnas pathway</td>
<td>Lipopolysaccharides, glycogen</td>
</tr>
<tr>
<td>Fructose-6-P</td>
<td></td>
<td>Cell wall constituents</td>
</tr>
<tr>
<td>Glyceraldehyde-3-P</td>
<td></td>
<td>Lipids</td>
</tr>
<tr>
<td>Glycerate-3-P</td>
<td></td>
<td>Cys, Gly, Ser</td>
</tr>
<tr>
<td>Phosphoenolpyruvate</td>
<td></td>
<td>Trp, Tyr</td>
</tr>
<tr>
<td>Pyruvate</td>
<td></td>
<td>Ala, Leu, Ile, Lys, Val</td>
</tr>
<tr>
<td>Acetyl-coenzyme A</td>
<td></td>
<td>Lipids, Leu</td>
</tr>
<tr>
<td>Erythrose-4-P</td>
<td>Pentose phosphate pathway</td>
<td>Phe, Trp, Tyr</td>
</tr>
<tr>
<td>Ribose-5-P</td>
<td></td>
<td>Nucleotides, His, Phe, Trp</td>
</tr>
<tr>
<td>2-Ketoglutarate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinyl-coenzyme A</td>
<td>Tricarboxylic acid cycle</td>
<td></td>
</tr>
<tr>
<td>Oxaloacetate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The information on the fate of each metabolite is derived from Noor et al. [96]. Note that the shading for each of the metabolic pathways indicated is the same as depicted in Figure 2.

provide endurance to oxidative stress [37]. Other cellular processes that consume NADPH include the recycling of methionine sulfoxides, oxidized forms of methionine known to result in protein deactivation, membrane damage, and cell ageing [74]. In particular, the major dehydrogenases in the EDEMP cycle of strain KT2440, such as glucose-6-P dehydrogenase (Zwf), are directly coupled to the formation of NADPH [75], with a small degree of promiscuity in cofactor selectivity [68**]. In a linear ED pathway, one NADPH molecule is generated per molecule of glucose-6-P entering the process through Zwf. In the EDEMP cycle, in contrast, the yield of NADPH can increase significantly depending on the extent of recycling (Figure 3). The importance of Zwf in P. putida is manifested by the presence of three isozymes: Zwf-1 (PP_1022), Zwf-2 (PP_4042), and Zwf-3 (PP_5351). Despite multiple attempts, a mutant lacking the three isoenzymes could not be obtained so far, perhaps as an indication of the essential character of this reaction. The NADPH balance in P. putida KT2440 growing on glucose is characterized by a slight catabolic overproduction of reducing power; however, the extent of cycling through the EDEMP cycle depends on specific environmental conditions. Specifically, the amount of NADPH formed depends on the partition of glucose between phosphorylation and oxidation (Figure 3). Such flexibility allows for the implementation of metabolic engineering strategies altering the overall redox balance of the cell, for example, moving the lifestyle of P. putida from aerobiosis to anoxia [76,77,78].

**Beyond Pseudomonas: the quest for the perfect bacterial chassis**

The physiological and metabolic properties discussed in the preceding sections argue for the selection of Pseudomonads as one starting point for constructing reliable SynBio platforms. Yet, the field of microbial cell factories is rapidly moving forward and the quest for the optimal
chassis (or a panel of different chassis based on other microorganisms) continues. To this end, there are some issues that are very often overlooked — potentially affecting not only Pseudomonas — and that should frame the way metabolic engineers program any given cell factory. Some of such issues are briefly enunciated below.

A first challenge is cell aging. The rosy picture of microbial cell factories is that they remain stable for a long time, running a maintenance metabolism with a grafted SynBio implant that results in production of a metabolite or material of industrial interest. Alas, besides the inevitable carboxylation of proteins due to oxidative stress in vivo [79], the simple passing of time conduces to cell ageing and an alteration of their biocatalytic performance [56,80]. Specifically, proteins age because of intrinsic clocks mediated by aspartate and asparagine residues (among other mechanisms [81,82]), making them to aggregate or to be cleaved by proteases [83–85] — particularly under stressful conditions [86]. A detailed exploration of this
Impact of carbon recycling on energy and redox balances. The action of the EDEMP cycle is illustrated with a theoretical example of flux distribution. The total carbon uptake is arbitrarily considered to be 100 (arbitrary units), and glucose can be split into the phosphorylative (f) or oxidative (x) branches, such that f + x = 100. When the system is at a steady state, the net formation rates of pyruvate, ATP, and NADPH adopt the values indicated in the inset table, r being the amount of triose phosphates recycled to hexose phosphates (i.e., recycling flux). The calculations were made on the assumptions that [i] gliceraldehyde-3-P dehydrogenase is NAD+ dependent and glucose-6-P dehydrogenase is NADP+ dependent, [ii] pyruvate is the end product of the lower Embden-Meyerhof-Parnas and Entner-Doudoroff glycolytic pathways, and [iii] the flux through the 2-ketogluconate loop (which uses NADPH as a cofactor to finally yield 6-phosphogluconate) represents a negligible fraction of the glucose consumed. Abbreviations are as indicated in the legend to Figure 2. Pi, inorganic phosphate.

A second question (and possibly a general bottleneck for metabolic engineering) is the spatial organization of bacterial metabolism [87**,88,89]. During normal growing conditions, a cell needs to prepare to build up a similar (i.e., daughter) structure. In an industrial setup, this situation is even more relevant, for example, in a continuous cultivation in which bacteria are kept growing exponentially. Since the source of genetic information is located in the chromosomal DNA, there should exist some link between the 3D organization of the genes, the location of their products, and the connectivity between the enzymes within a pathway [90]. The dichotomy soft-or-hard structures may endow cells with the ability to enter a control layer based on what one could call molecular fluidics, akin to microfluidics but ruled by a different physics. Fluidic channels could form not by assembly of physical pipes but by liquefaction of parts of a glass-like structure [91], or even by an asymmetric distribution of water (and therefore, metabolites) within the cell. Metabolism, again, lies at the core of this type of process: ca. 70% of water in the cell results come from biochemical reactions. Many of these reactions are actually driven by water usage (e.g., hydrolysis) or production (e.g., polymerization of macromolecules). This situation can be manipulated to boost the formation of a metabolite by considering the physical space at which the molecule is produced and consumed. Again, these are features of metabolic engineering that tend to be ignored in most whole-cell biocatalysis designs.

Finally, and in close connection with the concept of metabolic channeling in the bacterial cytoplasm, one could envision a setup in which the actual macroscopic shape of the cells (or their social behavior) is modified to enhance a given catalytic output, a trait that qualifies as synthetic morphology [92,93]. By targeting, for instance, the ability of cells to aggregate in biofilms, one could boost the biochemical properties of individual bacterial cells, such as the biodegradation of a target molecule [94*]. These artificially-designed spatial arrangements can be considered an integral part of the design of the ultimate physical SynBio chassis for biocatalysis [95].

SynBio has enriched the field of metabolic engineering not only by providing a wealth of genome-editing tools, DNA assembly methods, and modeling of complex biochemical networks, but also by pinpointing new fundamental questions and technological opportunities that will keep the community busy for quite a few years to come. The issues enumerated in the last section of this review are of special interest as they have been mostly neglected in most classical metabolic engineering designs, and some progress is expected in the near future. In particular, new model bacteria will provide mechanistic insights to these problems from a different perspective than in well-established platforms such as E. coli.

Conflict of interest
The authors declare that there are no conflicts of interest.

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:
- of special interest
- of outstanding interest


A superb account of the most recent advances in applied SynBio (including state-of-the-art methodologies, such as multiplexed genome engineering) for the targeted production of complex biochemical compounds.


An interesting analysis of the currently-available reduced genome microbial platforms. The non-essential components found in some essential genomes is addressed, which suggests that the design principle of minimal genomes should be reconsidered.


Whole-genome design and complete chemical synthesis were used to minimize the extent 1078-kb synthetic genome of Mycoplasma mycoides JCVI-syn1.0 to give rise to M. mycoides JCVI-syn3.0, which has a genome (531-kb long, spanning 473 genes) smaller than that of any autonomously replicating cell found in Nature.


Using a proteome resource allocation model, the authors show that overflow metabolism in E. coli is a rather global physiological response to cope with environment-dependent proteome demands of energy biogenesis and biomass synthesis.


The genome of the type strain KT2440 has been re-sequenced and re-annotated to further expand the current genomic-based knowledge of P. putida. About 242 new protein-coding genes were identified and the functions of 1548 genes were re-annotated. Specific catabolic pathways for 92 compounds (carbon, nitrogen and phosphorus sources) that could not be accommodated by extant genome-wide metabolic models were also predicted.


The aromatic polymer lignin, present in plant cell walls along cellulose, is proposed in this work as an alternative and cheap carbon source by using wild-type Pseudomonas putida KT2440 as the biocatalyst for the production of medium-chain-length polyhydroxyalkanoates.


Sulfoquinovose, the main source of organosulfur found in Nature, is an abundant compound in the rhizosphere. The authors describe a dedicated Entner–Doudoroff pathway for sulfoquinovose degradation, a striking example of the wealth of specific metabolic activities found in Pseudomonas species.  

68. Nikel PI, Chavarria M, Fuhrer T, Sauer U, de Lorenzo V: Pseudomonas putida KT2440 strain metabolizes glucose through a cycle formed by enzymes of the Entner–Doudoroff,
Embden–Meyerhof–Parnas, and pentose phosphate pathways. J Biol Chem 2015, 290:25920-25932. This work exposed the cyclic nature of glycolysis in strain KT2440 by adopting a multi-omic approach, opening new avenues for the implementation of metabolic engineering strategies aimed at modifying not only the central carbon metabolism but also for manipulating the redox balance in Pseudomonas.


The boundaries of redox and energy homeostasis in strain KT2440 are systematically analyzed in this study by altering the NADH and ATP regeneration rates. The authors showed that P. putida exhibits a rather robust metabolism that can accommodate these perturbations, which is in contrast with the situation in E. coli or yeast cells.


Bacterial metabolism is shaped by chemical constraints on the material and dynamic layout of enzymatic networks, the nature of which is disclosed by the authors discussing the physical structure of existing biosystems, ultimately stemming from the need to restrain chemical damage and limit the waste inherent to basic metabolic functions.


A first case example on how the spatial morphology of Pseudomonas putida can be altered to delivered a specific catalytic activity — in this case, by enhancing the biodegradation of a linear halokane.
