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2 **Industrial biotechnology of *Pseudomonas putida* and related species**

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22 **Abstract**

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1 Since their discovery many decades ago, *Pseudomonas putida* and related subspecies
2 have been intensively studied with regard to their potential application in industrial
3 biotechnology. Today, these gram-negative soil bacteria, traditionally known as well-
4 performing xenobiotic degraders are becoming efficient cell factories for various
5 products of industrial relevance including a full range of unnatural chemicals. This
6 development is strongly driven by systems biotechnology, integrating systems metabolic
7 engineering approaches with novel concepts from bioprocess engineering, including
8 novel reactor designs and renewable feedstocks.

9

10 **Keywords**

11 *Pseudomonas putida*, cell factory, bio-catalysis, biofilm, systems metabolic engineering,
12 synthetic biology, bioeconomy

1 Introduction

2 *Pseudomonas putida* is a Gram-negative rod-shaped bacterium occurring in various
3 environmental niches, due to its metabolic versatility, and low nutritional requirements
4 (Timmis 2002). Initiated by the pioneering discovery of its high capability to degrade
5 rather recalcitrant and inhibiting xenobiotics, extensive biochemical analysis of this
6 bacterium has been carried out in the recent years. In addition, *P. putida* shows a very
7 high robustness against extreme environmental conditions such as high temperature,
8 extreme pH or the presence of toxins or inhibiting solvents. Additionally, it is genetically
9 accessible and grows fast with simple nutrient demand (Martins Dos Santos et al.
10 2004). Meanwhile, *P. putida* is successfully used for the production of bio-based
11 polymers and a broad range of chemicals, far beyond its initial purpose for the
12 degradation of various toxic compounds. The sequencing of its genomic repertoire
13 (Nelson et al. 2002) and genome-wide pathway modeling (Puchalka et al. 2008) now
14 provide novel possibilities to further engineer this bacterium into a flexible cell factory for
15 bio-industrial application. Hereby, different species of *P. putida* vary to some extent in
16 their genetic repertoire and phenotypic behavior creating a high range of industrial
17 application possibilities. This review highlights fundamental aspects of the cellular
18 physiology of *P. putida* together with recent achievements in systems biology and
19 systems metabolic engineering.

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1 **Carbon core metabolism of *Pseudomonas putida***

2 Of particular interest for industrial application of *P. putida* are the central routes of
3 carbon metabolism, receiving carbon from the various converging pathways of substrate
4 utilization and supplying building blocks, cofactors and energy for the added-value
5 products of interest. It is interesting to note that *P. putida* differs in key aspects from the
6 generally much conserved central catabolic pathways of many other prokaryotic cells,
7 making its pathway repertoire and usage quite unique (Figure 1). Its fast growth, high
8 biomass yield and low maintenance demands are additional features important for
9 industrial application (Table 1).

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11 **Substrate uptake.** In contrast to various other industrial microorganisms, including e.g.
12 *E. coli*, *C. glutamicum* or *B. subtilis*, glucose is not the preferred carbon substrate for
13 pseudomonads. In the presence of succinate and other intermediates of the
14 tricarboxylic acid (TCA) cycle, carbon catabolic repression suppresses the assimilation
15 of glucose (Wolff et al. 1991). It is interesting to note that also some of the mechanisms
16 of substrate up-take vary from those of other bacteria. Differing from the typically
17 observed phosphoenolpyruvate-carbohydrate phosphotransferase system (PTS), *P.*
18 *putida* assimilates glucose by facilitated diffusion via the specific porin OprB.
19 Concerning the use of industrial substrates, *P. putida* is capable to use raw glycerol, a
20 technical by-product from the biodiesel industry (Ciesielski et al. 2010). Naturally, it
21 cannot grow on carbon five sugars such as D-xylose or L-arabinose, but has been
22 recently engineered towards utilization of these sugars (Meijnen et al. 2008; Meijnen et
23 al. 2009), important as major constituents of lignocellulosic biomass (Lee 1997),

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2**Catabolic metabolism.** Most strikingly, *P. putida* lacks a functional Embden-Meyerhof-
3Parnas (EMP) pathway due to the absence of a gene for 6-phosphofructokinase (*pfk*)
4along with the expression of several transcriptional regulators e.g. HexR (del Castillo et
5al. 2008). For the catabolism of sugars the Entner-Doudoroff pathway is employed
6instead (Fuhrer et al. 2005). This catabolic route generates two carbon three blocks, i.e.
7glyceraldehyde 3-phosphate and pyruvate, from break-down of sugars such as glucose.
8The pentose phosphate pathway, however, only operates in an anabolic mode to
9generate biomass precursors (Ebert et al. 2011). Once generated, pyruvate enters the
10so-called pyruvate shunt which yields either oxaloacetate or acetyl-CoA (Del Castillo et
11al. 2007). In addition to sugars and TCA cycle intermediates, *P. putida* KT2440 can
12metabolize a number of other substrates including fatty acids, polyols such as glycerol,
13amino acids, and aromatic compounds. The glyoxylate shunt, active in *P. putida*
14KT2440, is one of the anaplerotic reactions within the metabolic network. (Ebert et al.
152011).

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17**Redox metabolism.** The intracellular glucose provides a huge flexibility for *P. putida* to
18channel the sugar into different pathways. First, glucose can be either phosphorylated
19to glucose-6-phosphate, followed by NADPH coupled oxidation to 6-phosphogluconate
20to enter into the central energy catabolism. Alternatively, glucose can be subjected to
21successive oxidation steps which produce gluconate and 2-ketogluconate, respectively,
22whereby these two intermediates can be either secreted or phosphorylated to 6-
23phosphogluconate and 2-keto-6-phosphogluconate. The latter can be then also reduced

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1to 6-phosphogluconate so that a complex network of alternative pathways is formed. In
2*P. putida* KT2440 all three possible route towards 6-phosphogluconate work
3simultaneously (Del Castillo et al. 2007). The derived 6-phosphogluconate is the key
4intermediate of the major catabolic route in pseudomonads, the Entner-Doudoroff
5pathway. It should be noted that the network of glucose catabolism with multiple
6reduction and oxidation steps is efficient the supply of redox power, an important feature
7for whole cell applications in industrial bio-catalysis. The challenge of an elevated
8NADH oxidation rate did not affect the metabolic performance of *P. putida* KT2440
9NADH oxidation rate (Ebert et al. 2011), taking the specific substrate uptake rate as a
10measure. This differs from e.g. *E. coli* and *S. cerevisiae*, underscoring the remarkable
11potential of *P. putida* as a suitable strain for efficient NADH-demanding production of
12chemicals.

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14**Bio-conversion and degradation of non-natural chemicals.** With regard to its
15environmental applications, huge interest focused on the degradation pathways and the
16underlying mechanisms from early on (Nakazawa and Yokota 1973). In summary, five
17major degradation pathways have been found and characterized in *Pseudomonas*
18strains, namely; the β -ketoadipate, the phenylacetyl-CoA, the homogentisate, the
19gentisate, and the homo-*proto*-catechuate pathway, respectively. Hereby, *P. putida*
20shows a naturally high capacity to tolerate and modify aliphatic, aromatic and
21heterocyclic compounds (Schmid et al. 2001). In different isolates, plasmids could be
22identified which mediate the genes for the break-down of toluene (3-methyl-benzoate)
23(Nakazawa and Yokota 1973), naphthalene (Dunn and Gunsalus 1973), 4-

1chloronitrobenzene (Zhen et al. 2006), 2,4- xyleneol (Dean et al. 1989) or phenol
2(Herrmann et al. 1987). In addition, also chromosomal elements contribute to the
3degradation of aromatics (Jimenez et al. 2002).

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5Genetic engineering

6Molecular genetics has enabled the investigation of bacterial phenotypes and pathway
7manipulation for biotechnological applications in *P. putida* (Reva et al. 2006b). The
8genetic amenability of this bacterium has been proven to be large, making it suitable for
9metabolic engineering towards the creation of superior strains. It is an ideal host for
10heterologous gene expression (Meijnen et al. 2008; Ronchel et al. 1998) and has been
11certified as first Host-Vector Biosafety strain (HV1) which can be released into the
12environment. The Tn5-derived mini-transposon system has been the method of choice
13for genomic integration of DNA fragments in *P. putida* (De Lorenzo 1994; De Lorenzo et
14al. 1990). This straightforward technology allows several insertions into the same cell
15(De Lorenzo et al. 1998) and has recently led to the valuable creation of a genome-wide
16mutant library of *P. putida* KT2440 (Duque et al. 2007) as well as to the streamlining of
17the *P. putida* genome by using a combinatorial deletion method based on mini-
18transposon insertion and Flp-FRT recombination (Leprince et al. 2011). With regard to
19industrial application mini-transposons are less suitable due to the antibiotic markers
20often used. This can be overcome by a novel method, that is I-SceI based chromosomal
21engineering (Martinez-Garcia and de Lorenzo 2011) which enables the precise deletion
22of multiple genomic segments in *P. putida*.

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2In silico modeling of metabolism

3Driven by the sequencing of the genome of *P. putida* KT2440 (Nelson et al. 2002), and
4other species including *P. putida* Idaho (Tao et al. 2011), *P. putida* B6-2 (Tang et al.
52011) or *P. putida* S16 (Yu et al. 2011), the recent years have seen a huge progress in
6genome scale modeling of this bacterium (Nogales et al. 2008; Puchalka et al. 2008;
7Sohn et al. 2010). Varying to some extent in complexity and study focus, the created
8models display valuable information for systems level analysis of *P. putida* (Table 2),
9including investigation of the pathway repertoire, gene essentiality, resource distribution
10as well as model based strain design for the production of PHA (Puchalka et al. 2008).
11Model based predictions of in silico phenotypes were recently refined by the integration
12of experimental data on stoichiometric demands for anabolism and cellular maintenance
13which are crucial to increase the predictive power of computational design. This
14involved exact measurement of maintenance coefficients using a mini-scale chemostat
15system (Ebert et al. 2011) as well as growth dependent measurement of cellular
16composition (van Duuren 2011) (Table 2). These data were found useful to evaluate in
17silico gene essentiality as compared to gene expression, hereby overcoming
18inconsistencies from previous models. In addition, recent modeling approaches have
19aimed at resolving the dynamic behavior of pathways in *P. putida* and their regulation
20providing a new, interesting view into its metabolism (Koutinas et al. 2011; Koutinas et
21al. 2010; Silva-Rocha et al. 2011).

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1 Systems-level profiling by omics technologies

2 The development of quantitative omics technologies has provided a solid basis for
3 systems-wide analysis of metabolic and regulatory features of *P. putida*. Most of these
4 studies so far focused on xenobiotic degradation. However, recent examples have
5 initiated the investigation of this bacterium for the production of value-added chemicals.
6 This also includes a first set of multi-omics studies, combining systems profiling on
7 various levels towards superior strains (Verhoef et al. 2010).

8 **Transcriptomics.** Fascinating insights into the cellular program of *P. putida* during
9 xenobiotic degradation could be obtained by transcription profiling. A pioneering study
10 on the response of *P. putida* KT2440 (pWW0) to aromatic compounds (Dominguez-
11 Cuevas et al. 2006) revealed that toluene acts as a stressor rather than as a nutrient,
12 activating stress tolerance genes with the minimum expenditure of energy. Similarly,
13 other studies unraveled the stress response of the cell triggered by different toxic
14 agents (Del Castillo and Ramos 2007; Miyakoshi et al. 2007; Reva et al. 2006a; Yeom
15 et al. 2010) or the functionality of several regulators involved in this process (Fonseca et
16 al. 2008; Hervas et al. 2008; Morales et al. 2006; Moreno et al. 2009; Renzi et al. 2010).
17 More recently, transcriptomics was applied to identify targets in the strain *P. putida* S12
18 producing p-hydroxybenzoate on glucose and glycerol as carbon sources (Verhoef et
19 al. 2010).

20 **Proteomics.** Almost ten years ago, first studies created a comprehensive proteome
21 map for *P. putida* KT2440 and identified about 200 polypeptides using conventional 2D-
22 PAGE and matrix-assisted laser desorption ionization time-of-flight mass spectrometry

1(Heim et al. 2003). Complementary to the progress on the level of gene expression, the
2stress response of *P. putida* to different toxins was assessed also on the proteomic
3level (Krayl et al. 2003), so that we have a good knowledge on proteins involved in
4membrane composition, stabilization, detoxification or energy production (Benndorf et
5al. 2006; Santos et al. 2004; Segura et al. 2005; Volkers et al. 2006). In addition, studies
6included the characterization of aromatic degradation pathways and underlying
7mechanisms (Kim et al. 2006; Tsirogianni et al. 2006). More recently, also
8biotechnological production processes were characterized. This involved a study on the
9proteome of the strain *P. putida* CA-3 during growth on styrene under conditions of pure
10growth and also polyhydroxyalkanoate accumulation (Nikodinovic-Runic et al. 2009).
11Via shotgun proteomics it was possible to highlight the dual participation of proteins in
12stress response and PHA synthesis. *P. putida* S12 was investigated quite intensively
13with regard to the effects of the enhanced production of building block chemicals on
14cellular physiology and regulation (Verhoef et al. 2010; Wierckx et al. 2009; Wierckx et
15al. 2008).

16**Metabolomics.** Probably the most crucial part in quantitative metabolomics, that is the
17elucidation of pool sizes of the large set of intracellular metabolites, is related to
18sampling. In this regard, different sampling methods were tested and validated for their
19applicability to different catabolic and anabolic pathways in *P. putida* (Bolten et al.
202007). More recently, a comprehensive metabolome study of *P. putida* S12 revealed
21only minor differences among the pool size of all synthesized metabolites while growing
22in different carbon sources e. g. fructose, glucose, gluconate or succinate (Van Der
23Werf et al. 2008). Large differences, however, could be detected among specific

1metabolites belonging to the intermediary degradation routes of the carbon sources
2studied.

3**Fluxomics.** On the level pathway fluxes, most close to the phenotype, different ¹³C flux
4studies provided valuable insights into the carbon core metabolism of *P. putida*. For
5glucose-grown cells it was found that the Entner-Doudoroff pathway was the exclusive
6catabolic route, whereas the pentose phosphate pathway served mainly biosynthetic
7functions, involving the non-oxidative branch (Fuhrer et al. 2005). A more detailed view
8into the glucose catabolism in *Pseudomonas putida* revealed the simultaneous
9operation of three pathways that converge at the level of 6-phosphogluconate, which is
10then converted by the Entner-Doudoroff pathway to metabolites belonging the central
11metabolism (Del Castillo et al. 2007).With regard to the TCA cycle, most of the
12oxaloacetate was provided by the pyruvate shunt rather than by the direct oxidation of
13malate by malate dehydrogenase. At the level of substrate mixtures, *P. putida* exhibits a
14distinct pattern of internal carbon distribution as shown for simultaneous use of glucose
15and toluene (Del Castillo and Ramos 2007). Interestingly, toluene directs a larger
16amount of carbon than glucose into the TCA cycle, indicating the in vivo control by
17carbon catabolite repression that toluene exerts over glucose. The central carbon and
18energy metabolism of solvent tolerant *P. putida* DOT-T1E responded to the drastically
19increased the energy demands in the presence of toxic solvents and alcohols by an
20enhanced NAD(P)H regeneration (Blank et al. 2008a; Rühl et al. 2009). This was
21mediated by an increased specific glucose uptake rate together with a reduced anabolic
22demand and displays a major feature of this bacterium for industrial applications such
23as whole-cell redox bio-catalysis. More recently, metabolic flux analysis provided

1valuable information during metabolic engineering of a phenol overproducer strain
2derived from *P. putida* S12 (Wierckx et al. 2008) (Wierckx et al. 2009). Beyond
3experimental applications, flux patterns were also applied to validate mathematical
4models (Puchalka et al. 2008). In general there was a good agreement between in vivo
5and in silico data, whereby certain discrepancies were attributed to non-optimal
6allocation of resources in growing cells, differing from the optimization function imposed
7in the in silico analysis.

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9**Industrial applications of *P. putida* strains**

10Stimulated by their excellent production properties and the advent of genetic
11engineering, *P. putida* and related strains have been applied and optimized to a
12meanwhile broad portfolio of industrial products, involving bio-based materials, as well
13as de novo synthesis and biotransformation of high value chemicals and
14pharmaceuticals (Table 3).

15**Bio-based materials**

16Among bio-based polymers, polyhydroxyalkanoates (PHA) comprise a large class of
17polyesters. Their excellent biodegradability and biocompatibility is interesting for
18applications in various areas including tissue engineering or eco-friendly packaging
19(Khanna and Srivastava 2005; Liu and Chen 2007). Depending on the cultivation
20conditions, polyhydroxyalkanoates are accumulated as carbon and energy storage by
21*P. putida* which has been widely exploited for their targeted biosynthesis in this

1organism (Hoffmann and Rehm 2004). Their material properties, that is elasticity,
2crystallinity or rigidness depend on the monomeric composition which can be precisely
3controlled by fermentation strategies (Albuquerque et al. 2011; Hoffmann and Rehm
42004; Sun et al. 2009) or by metabolic engineering (Liu and Chen 2007). A better
5understanding on the regulation and processes involved in polymer biosynthesis has
6enabled targeted metabolic and protein engineering approaches to improve production
7efficiency of tailor made PHAs (Rehm 2010). As example, the weakening of the
8competing β -oxidation pathway in the strain *P. putida* KT2442 by deletion of FadA and
9FadB, significantly increased overall production and supported the formation of medium-
10chain-length polymers (Liu and Chen 2007; Ouyang et al. 2007). Further metabolic
11engineering of the β -oxidation reactions has made possible to synthesis different kinds
12of homo-polymers such as poly(3-hydroxyhexanoate), poly(3-hydroxyheptanoate)
13(Wang et al. 2011) , and poly(3-hydroxydecanoate) (Liu et al. 2011), as well as a novel
14PHA containing thioester groups in the side chain, which make them suitable for tailored
15chemical modifications (Escapa et al. 2011). More recently, the substrate specificity of
16type II PHA synthase was modified by site-directed mutagenesis to accept short-chain-
17length building blocks for PHA production and further extend the product portfolio of this
18class of bio-based plastics (Yang et al. 2011). Fed-batch cultivation of *P. putida* GPO1
19demonstrated the feasibility of large-scale PHA production (Elbahloul and Steinbüchel
202009). High cell density cultures are also well established in *P. putida* KT2440 (Sun et
21al. 2006), enabling efficient accumulation of PHA during carbon-limiting exponential
22feeding (Sun et al. 2007). A remarkable step towards straightforward biopolymer
23recovery has been made via a programmed self-disrupting *P. putida* strain that should

1 significantly reduce the costs of the process (Martínez et al. 2011). This illustrates the
2 great potential of *P. putida* species for an economically attractive production of PHA
3 with diverse composition.

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5 **Bioconversion and de-novo synthesis of chemicals**

6 Beyond its more traditional application for xenobiotic degradation and PHA production,
7 *P. putida* is gaining more and more importance as host for whole cell bio-catalysis and
8 de novo synthesis of chemicals (Table 3). This takes significant benefit from its well-
9 known capacity to tolerate and modify aliphatic, aromatic and heterocyclic compounds
10 related to a versatile enzymatic set of mono-oxygenases, di-oxygenases and
11 hydroxylases (Schmid et al. 2001). *P. putida* KT2440 is able to metabolize benzoate
12 which gives access to various interesting intermediates of the degradation route
13 (Jiménez et al. 2002). As example, *cis-cis* muconate, an aromatic intermediate of
14 benzoate degradation, is a suitable precursor for the production of adipic acid opening
15 novel routes towards nylon-6,6 (Draths and Frost 1994). Recently, the generation of a
16 (*catR*) deficient mutant of KT2440 showed high rate and yield of *cis-cis* muconate from
17 the co-metabolization of benzoate in the presence of glucose (Van Duuren et al. 2011c).
18 This overproducer strain was used in a pH-stat fed-batch process which resulted in high
19 specific productivity (Van Duuren et al. 2011b) and promising life cycle characteristics
20 for different feed stocks (Van Duuren et al. 2011a). Hereby, *P. putida* exhibits rather
21 high tolerance as compared to many other industrial production organisms. This
22 appears as an excellent starting point to overcome a major bottleneck, that is, substrate

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1and product toxicity to the often unnatural chemical compounds. Indeed, solvent-
2tolerant strains of *P. putida* were successfully used for stereospecific epoxidation of
3styrene by the strain DOT-T1E (Blank et al. 2008b), o-cresol formation from toluene with
4*P. putida* T-57 (Faizal et al. 2005) or de novo synthesis of p-coumarate (Nijkamp et al.
52007), p-hydroxybenzoate (Verhoef et al. 2007) or p-hydroxystyrene (Verhoef et al.
62009), all using *P. putida* S12. These successful developments also recruited metabolic
7engineering strategies. Reprogramming of the p-coumarate producing strain S12 for p-
8hydroxy-styrene production involved introduction of the genes *pal* and *pdc* encoding L-
9phenylalanine/L-tyrosine ammonia lyase and p-coumaric acid decarboxylase,
10respectively. Degradation of the p-coumarate intermediate was prevented by
11inactivating the *fcs* gene encoding feruloyl-coenzyme A synthetase (Verhoef et al.
122009). In addition novel reactor configurations have been developed in recent years,
13showing advantage with regard to toxicity effects. This comprised two-phase-liquid-
14liquid-cultivation systems with an aqueous and an organic phase (Schmid et al. 2001;
15Verhoef et al. 2009; Wierckx et al. 2005). Beyond this, alternative concepts involving
16catalytically active biofilms came into focus because of their inherent characteristics of
17self-immobilization, high resistance to reactants and long-term activity, which all facilitate
18continuous processing (Rosche et al. 2009). Using the biofilm-forming and engineered
19*Pseudomonas* strain VLB120 Δ C, a stable and highly efficient continuous (S)-styrene
20oxide production process was recently established (Gross et al. 2010) and optimized
21(Halan et al. 2010). This profited from a high volumetric productivity in situ substrate
22feed and product recovery (Halan et al. 2010), and improved tolerance and robustness
23as compared to planktonic cultures (Halan et al. 2011).

2Pharmaceuticals and agrochemicals

3Natural products of microbial origin are widely used as pharmaceuticals and in agro-
4chemistry. These compounds are often biosynthesized by multifunctional mega-
5synthetases whose genetic engineering and heterologous expression offer considerable
6promise, especially if the natural hosts are genetically difficult to handle, slow growing,
7unculturable, or even unknown (Wenzel et al. 2005). In this regard, *P. putida* has shown
8to be capable to express and activate biosynthetic proteins of complex natural products
9from myxobacteria (Gross et al. 2005; Gross et al. 2006; Wenzel et al. 2005). This has
10enabled production of high-value pharmaceuticals in this bacterium (Table 3). Using *P.*
11*putida* KT2440 as heterologous host, myxochromide S production was successfully
12established (Stephan et al. 2006). Specific feeding strategies unraveled metabolic
13bottlenecks for the supply of myxochromid building blocks suggesting process
14optimization by metabolite feeding (Stephan et al. 2006) or metabolic engineering
15toward improved supply of the required compounds. In addition *P. putida* FG2005 was
16used as production host for heterologous myxothiazol production (Gross et al. 2006).
17More recently, transposition was established for genetic engineering of *P. putida*
18allowing efficient transfer of extremely large gene clusters which in general build the
19basis for biosynthesis of natural products (Fu et al. 2008). In another study, the insertion
20heterologous genes into *P. putida* KT2440 allowed the biosynthesis of valuable
21carotenoids such as zeaxanthin (Beuttler et al. 2011). Hence, previous size problems
22for transformation of *P. putida* appear to be solved and offer novel possibilities to further
23exploit *P. putida* as production platform for high-value natural products.

2Conclusions and future perspectives

3Similar to other industrial microorganisms, the product portfolio of *P. putida* has strongly
4evolved in recent years (Table 3). Its good genetic accessibility and the naturally high
5tolerance appear as desirable features to overcome the toxic and harsh conditions
6typically linked to industrial bio-catalysis and de-novo synthesis of often unnatural
7chemicals. It interesting to note, that quite a few commercial processes in biotechnology
8are based on this versatile bacterium (Table 4). *P. putida* and its enzyme repertoire are
9involved in the industrial synthesis of chiral compounds (Hermes et al. 1993; Schulze
10and Wubbolts 1999), paclitaxel (Patel et al. 1994), 5-methylpirazine-2-carboxylic acid
11(Kiener 1992) among others, involving large chemical and biotechnological companies
12such as Pfizer (USA), Lonza (Switzerland), DSM (The Netherlands), DuPont (USA), or
13BASF (Germany) (Schulze and Wubbolts 1999). The application range of *P. putida* in
14industrial biotechnology has good chances to further grow and expand in the future
15considering the interesting pipeline of novel products becoming available via efficient *P.*
16*putida* cell factories. Interesting future developments might consider the integration of *P.*
17*putida* into existing or currently developed pipelines of utilizing renewable feedstocks or
18industrial wastes for sustainable bio-production as visualized in Figure 2. The rich
19intrinsic pathway repertory enables *P. putida* to degrade and metabolize a broad range
20of compounds, including also complex aromatics. Together with its natural high
21tolerance to harsh and toxic conditions this seems beneficial to couple *P. putida* to
22various streams of renewable feedstocks. As example, lignocellulosic biomass from
23catalytic pyrolysis containing also aromatic compounds (Bu et al. 2011) could be

1 converted by *P. putida* capable to utilize such substrates into added value products
2 such as as *cis-cis* muconate, an excellent precursor for the synthesis of adipic acid.
3 Also in the sugar pipeline, *P. putida* seem valuable to create novel chemicals or
4 materials. Hereby, the naturally high tolerance of *P. putida* to harsh and toxic conditions
5 together with flexible genetic modifications displays an excellent starting point of further
6 developing it into a production platform for other, non-natural chemicals which are not
7 accessible so far. This will require experimental and computational systems level
8 strategies to disentangle the complexity of the *Pseudomonas putida* central and
9 peripheral metabolic pathways towards their targeted optimization. Synthetic biology will
10 add a next level of design space to reshape metabolism for enhanced bio-production,
11 e.g. via fine-modulated expression and control of regulation networks or the integration
12 of complex heterologous pathways Rational strain engineering will be further completed
13 by novel concepts or evolutionary engineering, boosting industrial implementation of
14 *Pseudomonas* strains via novel phenotypes with even enhanced tolerance to industrial
15 environments, a promising perspective for *P. putida*.

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4

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1 Table 1. Physiological growth data of *Pseudomonas putida* KT2440 on glucose as
 2 carbon source. The data comprise maximum specific growth rate (μ), biomass yield
 3 ($Y_{x/s}$) and maintenance coefficient (m_x) from batch as well as continuous culture

4

μ (h^{-1})	$Y_{x/s}$ ($g\ g^{-1}$)	m_x ($mmol\ g^{-1}\ h^{-1}$)	Reference
^a 0.81	-	-	(Sohn et al. 2010)
^a 0.73	^a 0.56	^b 0.062	(Ebert et al. 2011)
^b 0.59	^b 0.39	^b 0.171	(van Duuren 2011)

5

6

^a Batch culture

7

^b Chemostat culture

8

9

1Table 2. Genome scale models for *Pseudomonas putida* KT2440

2

3

N° of genes	N° of reactions	N° of metabolites	Reference
746	950	911	(Nogales et al. 2008)
815	877	888	(Puchalka et al. 2008)
900	1071	1044	(Sohn et al. 2010)
746	952	917	(van Duuren 2011)

4

1

2

1 Table 3: Overview on biotechnology products derived by natural and recombinant strains of *Pseudomonas putida*.

Product	Parent strain	Production strain	Comment	Reference
PHA hetero-polymer	KT2442	KT2442		Ouyang 2007
PHA hetero-polymer with modified monomer composition	KT2442	KTOY06	Altered β -oxidation	Ouyang 2007
PHA hetero-polymer with high-content of 3-hydroxytetradecanoate	KT2442	KTOY06		Liu 2007
PHA C ₆ and C ₇ homo-polymers	KT2442	KTHH03	Modified β -oxidation; feeding of hexanoate and heptanoate	Wang 2011
PHA C ₅ homo-polymer	KTHH03	KTHH08	Deletion of mcl PHA synthase genes (<i>phaC</i>), plasmid-based expression of the PHA synthesis operon (<i>phaPCJ</i>) from <i>Aeromonas hydrophila</i> ; valerate used as carbon source	Wang 2011
PHA C ₃ and C ₄ homo-polymer	KTHH03	KTHH06	Replacement of endogenous <i>phaC</i> by <i>phaC</i> from <i>Ralstonia eutropha</i> ; γ -butyrolactone as precursor	Wang 2011
Myxochromide S	KT2440	P.putida::CMch37a	Heterologous expression of myxochromide S biosynthetic gene cluster from <i>Stigmatella aurantiaca</i>	Stephan 2006
--	KT2440	FG2005	Integration of methyl-malonyl-CoA pathway from <i>Sorangium cellulosum</i>	Gross 2006
Myxothiazol	FG2005	FG2005::Pm-mta	Integration of myxothiazol synthesis cluster from <i>Stigmatella aurantiaca</i>	Gross 2006
p-Hydroxysterene	S12	S12 427 Δ fcs	Introduction of <i>pal</i> (<i>R. glutinis</i>) and <i>pdC</i> (<i>L. plantarum</i>), inactivating <i>fcs</i> genes	Verhoef 2009
D-Glucosaminic acid	GNA5	pJNTpalpdC GNA5	D-glucosaminic acid accumulated during oxidative fermentation process	Wu 2010
Phenol	S12	S12	Introduction of <i>tpl</i> gene from <i>Pantea agglomeran</i> , overexpression of the <i>aroF-1</i> gene	Wierckx 2005
p-Coumarate	S12	S12 C3	Inactivation of <i>fcs</i> , construction of phenylalanine auxotrophic mutant	Nijkamp 2007
3-Nitrocatechol	F1	F1	Process optimization for bio-catalytic production	Prakash 2010
3-Methylcatechol	F1 and F107	MC1 and MC2	F107 as a 3-methylcatechol accumulating mutant of F1 with expression of <i>todC1C2BAD</i> under control of an inducible regulatory region	Hüsken 2001
o-Cresol	T-57	TODD1	<i>todD</i> knockout mutant of T-57	Faizal 2005
Aliphatic alcohols	PpS81	PpS8141	Introduction of the <i>alk</i> regulon (<i>alkBFGH/alkST</i>) from <i>Pseudomonas oleovorans</i>	Bosetti 1992
4-Valerolactone	KT2440	KT2440	Introduction of <i>tesB</i> (<i>E. coli</i> G1655) to secrete 4-hydroxyvalerate and <i>pon1</i> (human paraoxonase I), expressed extra-cytosolic to catalyze the	Martin 2010

p-Hydroxybenzoate	S12	S12pal_xylB7	utilization the intermediate 4-hydroxyvalerate Additional copy of <i>aroF-1</i> gene, introduction of <i>xylAB_FGH</i> genes (<i>E.coli</i>) and the <i>pal</i> gene (<i>R.</i> <i>toruloides</i>)	Meijnen 2011
2-Alkyl-4(1H)-quinolones and related derivates	KT2440	KT2440 [pBBR- pqsABCD]	Insertion of <i>pqsABCD</i> from <i>P. aeruginosa</i>	Niewerth 2011
Styreneoxide	DOT-T1E	DOT-T1E [pTEZ240]	Heterologous expression of styrene monooxygenase StyAB from <i>P. spec.</i> VLB120	Blank 2008
4-Hydroxyquinaldine	KT2440	KT2440 (pKP1)	Inserted genes <i>qoxLMS</i> (<i>Arthrobacter</i> <i>nitroguajoccolicus</i> R61a)	Ütkür 2011
Biodesulfurization	S12	DS23	Insertion of desulfurizing gene cluster (<i>dszABCD</i>) (<i>P. putida</i> A4)	Tao 2011
Bioconversion of limonin	G7	G7	Use of whole cells permeabilized with EDTA and lysozyme	Malik 2011

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9Table 4. Industrial processes based on *Pseudomonas* strains

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Product	Biocatalyst	Applicability	Company	Source
2-quinoxalinecarboxylic acid	<i>P. putida</i> ATCC 33015	Biological activity	Pfizer (USA)	(Wong et al. 2002)
5-methylpirazine-2-carboxylic acid	<i>P. putida</i> ATCC 33015	Pharmaceutical	Lonza (Switzerland)	(Kiener 1992)
Chiral amines	<i>Pseudomonas</i> DSM 8246	Biological activity	BASF (Germany)	(Schulze and Wubbolts 1999)
5-cyanopentanamide	<i>P. putida</i>	Catalysis	DuPont (USA)	(Stieglitz et al. 1996)
(S)-2-Chloropropionoc acid	<i>Pseudomonas</i>	Herbicides	Astra Zeneca (USA)	(Schulze and Wubbolts 1999)
D- <i>p</i> -hydroxyphenyl glycine	<i>P. putida</i>	Pharmaceutical	Several companies	(Schulze and Wubbolts 1999)
Chiral compounds	<i>P. putida</i> ATCC 12633	Pharmaceutical	DSM (The Netherlands)	(Hermes et al. 1993)
4-[6-hydroxypyridin-3-yl]-4-oxobutyrate	<i>Pseudomonas</i> DSM 8653	Pharmaceutical	Lonza (Switzerland)	(Schmid et al. 2001)
Paclitaxel	<i>Pseudomonas</i> lipase AK	Pharmaceutical	Bristol-Myers Squibb	(Patel et al. 1994)

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2Figure Legends

3Figure 1. Metabolic pathways in the carbon core metabolism of *Pseudomonas putida*

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5Figure 2. Integration of *Pseudomonas putida* as cell factory in the bio-based production
6pipelines from renewable resources.

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