Industrial biotechnology of Pseudomonas putida and related species

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Abstract

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

Keywords

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

Introduction

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

Carbon core metabolism of *Pseudomonas putida*

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

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

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

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

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

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

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

Genetic engineering

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

In silico modeling of metabolism

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

Systems-level profiling by omics technologies

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

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

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

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

Metabolomics. Probably the most crucial part in quantitative metabolomics, that is the elucidation of pool sizes of the large set of intracellular metabolites, is related to sampling. In this regard, different sampling methods were tested and validated for their applicability to different catabolic and anabolic pathways in *P. putida* (Bolten et al. 2007). More recently, a comprehensive metabolome study of *P. putida* S12 revealed only minor differences among the pool size of all synthesized metabolites while growing in different carbon sources e. g. fructose, glucose, gluconate or succinate (Van Der Werf et al. 2008). Large differences, however, could be detected among specific

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

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

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

Industrial applications of *P. putida* strains

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

Bio-based materials

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

organism (Hoffmann and Rehm 2004). Their material properties, that is elasticity, crystallinity or rigidness depend on the monomeric composition which can be precisely controlled by fermentation strategies (Albuquerque et al. 2011; Hoffmann and Rehm 2004; Sun et al. 2009) or by metabolic engineering (Liu and Chen 2007). A better understanding on the regulation and processes involved in polymer biosynthesis has enabled targeted metabolic and protein engineering approaches to improve production efficiency of tailor made PHAs (Rehm 2010). As example, the weakening of the competing β-oxidation pathway in the strain *P. putida* KT2442 by deletion of FadA and FadB, significantly increased overall production and supported the formation of medium-chain-length polymers (Liu and Chen 2007; Ouyang et al. 2007). Further metabolic engineering of the β-oxidation reactions has made possible to synthesis different kinds of homo-polymers such as poly(3-hydroxyhexanoate), poly(3-hydroxyheptanoate) (Wang et al. 2011) , and poly(3-hydroxydecanoate) (Liu et al. 2011), as well as a novel PHA containing thioester groups in the side chain, which make them suitable for tailored chemical modifications (Escapa et al. 2011). More recently, the substrate specificity of type II PHA synthase was modified by site-directed mutagenesis to accept short-chain-length building blocks for PHA production and further extend the product portfolio of this class of bio-based plastics (Yang et al. 2011). Fed-batch cultivation of *P. putida* GPo1 demonstrated the feasibility of large-scale PHA production (Elbahloul and Steinbüchel 2009). High cell density cultures are also well established in *P. putida* KT2440 (Sun et al. 2006), enabling efficient accumulation of PHA during carbon-limiting exponential feeding (Sun et al. 2007). A remarkable step towards straightforward biopolymer recovery has been made via a programmed self–disrupting *P. putida* strain that should

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

Bioconversion and de-novo synthesis of chemicals

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

and product toxicity to the often unnatural chemical compounds. Indeed, solvent-tolerant strains of *P. putida* were successfully used for stereospecific epoxidation of styrene by the strain DOT-T1E (Blank et al. 2008b), o-cresol formation from toluene with *P. putida* T-57 (Faizal et al. 2005) or de novo synthesis of p-coumarate (Nijkamp et al. 2007), p-hydroxybenzoate (Verhoef et al. 2007) or p-hydroxystyrene (Verhoef et al. 2009), all using *P. putida* S12. These successful developments also recruited metabolic engineering strategies. Reprogramming of the p-coumarate producing strain S12 for p-hydroxy-styrene production involved introduction of the genes pal and pdc encoding L-phenylalanine/L-tyrosine ammonia lyase and p-coumaric acid decarboxylase, respectively. Degradation of the p-coumarate intermediate was prevented by inactivating the *fcs* gene encoding feruloyl-coenzyme A synthetase (Verhoef et al. 2009). In addition novel reactor configurations have been developed in recent years, showing advantage with regard to toxicity effects. This comprised two-phase-liquid-liquid-cultivation systems with an aqueous and an organic phase (Schmid et al. 2001; Verhoef et al. 2009; Wierckx et al. 2005). Beyond this, alternative concepts involving catalytically active biofilms came into focus because of their inherent characteristics of self-immobilization, high resistance to reactants and long-term activity, which all facilitate continuous processing (Rosche et al. 2009). Using the biofilm-forming and engineered *Pseudomonas* strain VLB120ΔC, a stable and highly efficient continuous (S)-styrene oxide production process was recently established (Gross et al. 2010) and optimized (Halan et al. 2010). This profited from a high volumetric productivity in situ substrate feed and product recovery (Halan et al. 2010), and improved tolerance and robustness as compared to planktonic cultures (Halan et al. 2011).

Pharmaceuticals and agrochemicals

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

Conclusions and future perspectives

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

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

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References

Albuquerque MGE, Martino V, Pollet E, AvErous L, Reis MAM (2011) Mixed culture polyhydroxyalkanoate (PHA) production from volatile fatty acid (VFA)-rich streams: Effect of substrate composition and feeding regime on PHA productivity, composition and properties. J Biotechnol 151:66-76

Benndorf D, Thiersch M, Loffhagen N, Kunath C, Harms H (2006) *Pseudomonas putida* KT2440 responds specifically to chlorophenoxy herbicides and their initial metabolites. Proteomics 6:3319-3329

Beuttler H, Hoffmann J, Jeske M, Hauer B, Schmid R, Altenbuchner J, Urlacher V (2011) Biosynthesis of zeaxanthin in recombinant *Pseudomonas putida*. App Microbiol Biotechnol 89:1137-1147

Blank LM, Ionidis G, Ebert BE, Buhler B, Schmid A (2008a) Metabolic response of *Pseudomonas putida* during redox biocatalysis in the presence of a second octanol phase. FEBS J 275:5173-5190

Blank LM, Ionidis G, Ebert BE, Bühler B, Schmid A (2008b) Metabolic response of *Pseudomonas putida* during redox biocatalysis in the presence of a second octanol phase. FEBS J 275:5173-5190

Bolten CJ, Kiefer P, Letisse F, Portais JC, Wittmann C (2007) Sampling for metabolome analysis of microorganisms. Anal Chem 79:3843-3849

Bu Q, Lei H, Ren S, Wang L, Holladay J, Zhang Q, Tang J, Ruan R (2011) Phenol and phenolics from lignocellulosic biomass by catalytic microwave pyrolysis. Bioresour Technol 102:7004-7007

Ciesielski S, Pokoj T, Klimiuk E (2010) Cultivation-dependent and -independent characterization of microbial community producing polyhydroxyalkanoates from raw glycerol. J Microbiol Biotechnol 20:853-861

De Lorenzo V (1994) Designing microbial systems for gene expression in the field. Trends Biotechnol 12:365-371

De Lorenzo V, Herrero M, Jakubzik U, Timmis KN (1990) Mini-Tn5 transposoon derivatives for insertion mutagenesis, promoter probing, and chromosomal insertion of cloned DNA in gram-negative eubacteria. J Bacteriol 172:6568-6572

De Lorenzo V, Herrero M, Sanchez JM, Timmis K (1998) Mini-transposons in microbial ecology and environmental biotechnology. FEMS Microbiol Ecol 27:211-224

Dean HF, Cheevadhanarak S, Skurray RA, Bayly RC (1989) Characterisation of a degradative plasmid in *Pseudomonas putida* that controls the expression of 2,4-xylenol degradative genes. FEMS Microbiol Lett 61:153-157

del Castillo T, Duque E, Ramos JL (2008) A Set of Activators and Repressors Control Peripheral Glucose Pathways in *Pseudomonas putida* To Yield a Common Central Intermediate. J Bacteriol 190:2331-2339

Del Castillo T, Ramos JL (2007) Simultaneous catabolite repression between glucose and toluene metabolism in *Pseudomonas putida* is channeled through different signaling pathways. J Bacteriol 189:6602-6610

Del Castillo T, Ramos JL, Rodriguez-Herva JJ, Fuhrer T, Sauer U, Duque E (2007) Convergent peripheral pathways catalyze initial glucose catabolism in *Pseudomonas putida*: Genomic and flux analysis. J Bacteriol 189:5142-5152

Dominguez-Cuevas P, Gonzalez-Pastor JE, Marques S, Ramos JL, De Lorenzo V (2006) Transcriptional tradeoff between metabolic and stress-response programs in Pseudomonas putida KT2440 cells exposed to toluene. J Biol Chem 281:11981-11991

Draths KM, Frost JW (1994) Environmentally compatible synthesis of adipic acid from D-glucose. J Am Chem Soc 116:399-400

Dunn NW, Gunsalus IC (1973) Transmissible plasmid coding early enzymes of naphthalene oxidation in *Pseudomonas putida*. J Bacteriol 114:974-979

Duque E, Molina-Henares AJ, Torre Jdl, Molina-Henares MA, Castillo Td, Lam J, Ramos JL, Ramos J-L, Filloux A (2007) Towards a Genome-Wide Mutant Library of *Pseudomonas putida* Strain KT2440. Pseudomonas. Springer Netherlands. p 227-251. Book

Ebert BE, Kurth F, Grund M, Blank LM, Schmid A (2011) Response of Pseudomonas putida KT2440 to increased NADH and ATP demand. Appl Environ Microbiol 77:6597-6605

Elbahloul Y, Steinbüchel A (2009) Large-scale production of poly(3-hydroxyoctanoic acid) by *Pseudomonas putida* GPo1 and a simplified downstream process. Appl Environ Microbiol 75:643-651

Escapa I, Morales V, Martino V, Pollet E, Averous L, Garcia J, Prieto M (2011) Disruption of B-oxidation pathway in *Pseudomonas putida* KT2442 to produce new functionalized PHAs with thioester groups. Appl Microbiol Biotechnol 89:1583-1598

Faizal I, Dozen K, Hong CS, Kuroda A, Takiguchi N, Ohtake H, Takeda K, Tsunekawa H, Kato J (2005) Isolation and characterization of solvent-tolerant *Pseudomonas putida* strain T-57, and its application to biotransformation of toluene to cresol in a two-phase (organic-aqueous) system. J Ind Microbiol Biotechnol 32:542-547

Fonseca P, Moreno R, Rojo F (2008) Genomic analysis of the role of RNase R in the turnover of *Pseudomonas putida* mRNAs. J Bacteriol 190:6258-6263

Fu J, Wenzel SC, Perlova O, Wang J, Gross F, Tang Z, Yin Y, Stewart AF, Müller R, Zhang Y (2008) Efficient transfer of two large secondary metabolite pathway gene clusters into heterologous hosts by transposition. Nucleic ac res 36.

Fuhrer T, Fischer E, Sauer U (2005) Experimental identification and quantification of glucose metabolism in seven bacterial species. J Bacteriol 187:1581-1590

Gross F, Gottschalk D, Müller R (2005) Posttranslational modification of myxobacterial carrier protein domains in Pseudomonas sp. by an intrinsic phosphopantetheinyl transferase. Appl Microbiol Biotechnol 68:66-74

Gross F, Ring MW, Perlova O, Fu J, Schneider S, Gerth K, Kuhlmann S, Stewart AF, Zhang Y, Müller R (2006) Metabolic Engineering of *Pseudomonas putida* for

Methylmalonyl-CoA Biosynthesis to Enable Complex Heterologous Secondary Metabolite Formation. Chem Biol 13:1253-1264

Gross R, Lang K, Bühler K, Schmid A (2010) Characterization of a biofilm membrane reactor and its prospects for fine chemical synthesis. Biotechnol Bioeng 105:705-717

Halan B, Schmid A, Buehler K (2010) Maximizing the productivity of catalytic biofilms on solid supports in membrane aerated reactors. Biotechnol Bioeng 106:516-527

Halan B, Schmid A, Buehler K (2011) Real-time solvent tolerance analysis of *Pseudomonas sp*. Strain VLB120ΔC catalytic biofilms. Appl Environ Microbiol 77:1563-1571

Heim S, Ferrer M, Heuer H, Regenhardt D, Nimtz M, Timmis KN (2003) Proteome reference map of *Pseudomonas putida* strain KT2440 for genome expression profiling: Distinct responses of KT2440 and *Pseudomonas aeruginosa* strain PAO1 to iron deprivation and a new form of superoxide dismutase. Environ Microbiol 5:1257-1269

Hermes HFM, Sonke T, Peters PJH, van Balken JAM, Kamphuis J, Dijkhuizen L, Meijer EM (1993) Purification and Characterization of an l-Aminopeptidase from *Pseudomonas putida* ATCC 12633. Appl Environ Microbiol 59:4330-4334

Herrmann H, Janke D, Krejsa S, Kunze I (1987) Involvement of the plasmid pPGH1 in the phenol degradation of *Pseudomonas putida* strain H. FEMS Microbiol Lett 43:133-137

Hervas AB, Canosa I, Santero E (2008) Transcriptome analysis of Pseudomonas putida in response to nitrogen availability. J Bacteriol 190:416-420

Hoffmann N, Rehm BHA (2004) Regulation of polyhydroxyalkanoate biosynthesis in *Pseudomonas putida* and *Pseudomonas aeruginosa*. FEMS Microbiol Lett 237:1-7

Jimenez JI, Miambres B, Garcia JL, Diaz E (2002) Genomic analysis of the aromatic catabolic pathways from *Pseudomonas putida* KT2440. Environ Microbiol 4(:824-841

Khanna S, Srivastava AK (2005) Recent advances in microbial polyhydroxyalkanoates. Proc Biochem 40:607-619

Kiener A (1992) Enzymatic Oxidation of Methyl Groups on Aromatic Heterocycles: A Versatile Method for the Preparation of Heteroaromatic Carboxylic Acids. Angewandte Chem Inter Edition Engl 31:774-775

Kim YH, Cho K, Yun SH, Kim JY, Kwon KH, Yoo JS, Kim SI (2006) Analysis of aromatic catabolic pathways in *Pseudomonas putida* KT2440 using a combined proteomic approach: 2-DE/MS and cleavable isotope-coded affinity tag analysis. Proteomics 6:1301-1318

Koutinas M, Kiparissides A, Lam MC, Silva-Rocha R, Godinho M, de Lorenzo V, Martins dos Santos VAP, Pistikopoulos EN, Mantalaris A (2011) Improving the prediction of *Pseudomonas putida* mt-2 growth kinetics with the use of a gene expression regulation model of the TOL plasmid. Biochem Eng J 55:108-118

Koutinas M, Lam MC, Kiparissides A, Silva-Rocha R, Godinho M, Livingston AG, Pistikopoulos EN, de Lorenzo V, Dos Santos VA, Mantalaris A (2010) The regulatory logic of m-xylene biodegradation by *Pseudomonas putida* mt-2

exposed by dynamic modelling of the principal node Ps/Pr of the TOL plasmid. Environ Microbiol 12:1705-1718

Krayl M, Benndorf D, Loffhagen N, Babel W (2003) Use of proteomics and physiological characteristics to elucidate ecotoxic effects of methyl tert-butyl ether in *Pseudomonas putida* KT2440. Proteomics 3:1544-1552

Lee J (1997) Biological conversion of lignocellulosic biomass to ethanol. J Biotechnol 56:1-24

Leprince A, Janus D, de Lorenzo V, Santos VMd, Weber W, Fussenegger M (2011) Streamlining of a *Pseudomonas putida* Genome Using a Combinatorial Deletion Method Based on Minitransposon Insertion and the Flp-*FRT* Recombination System. Synth Gene Networks. Humana Press. p 249-266. Book Chapter

Liu Q, Luo G, Zhou XR, Chen GQ (2011) Biosynthesis of poly(3-hydroxydecanoate) and 3-hydroxydodecanoate dominating polyhydroxyalkanoates by β-oxidation pathway inhibited *Pseudomonas putida*. Metabol Eng 13:11-17

Liu W, Chen GQ (2007) Production and characterization of medium-chain-length polyhydroxyalkanoate with high 3-hydroxytetradecanoate monomer content by fadB and fadA knockout mutant of *Pseudomonas putida* KT2442. Appl Microbiol Biotechnol 76:1153-1159

Martinez-Garcia E, de Lorenzo V (2011) Engineering multiple genomic deletions in Gram-negative bacteria: Analysis of the multi-resistant antibiotic profile of *Pseudomonas putida* KT2440. Environ Microbiol 13:2702-2716

Martínez V, García P, García JL, Prieto MA (2011) Controlled autolysis facilitates the polyhydroxyalkanoate recovery in *Pseudomonas putida* KT2440. Microb Biotechnol 4:533-547

Martins Dos Santos VAP, Heim S, Moore ERB, Strätz M, Timmis KN (2004) Insights into the genomic basis of niche specificity of *Pseudomonas putida* KT2440. Environ Microbiol 6:1264-1286

Meijnen JP, De Winde JH, Ruijssenaars HJ (2008) Engineering *Pseudomonas putida* S12 for efficient utilization of D-xylose and L-arabinose. Appl Environ Microbiol 74:5031-5037

Meijnen JP, De Winde JH, Ruijssenaars HJ (2009) Establishment of oxidative D-xylose metabolism in *Pseudomonas putida* S12. Appl Environ Microbiol 75:2784-2791

Miyakoshi M, Shintani M, Terabayashi T, Kai S, Yamane H, Nojiri H (2007) Transcriptome analysis of *Pseudomonas putida* KT2440 harboring the completely sequenced IncP-7 plasmid pCAR1. J Bacteriol 189:6849-6860

Morales G, Ugidos A, Rojo F (2006) Inactivation of the *Pseudomonas putida* cytochrome o ubiquinol oxidase leads to a significant change in the transcriptome and to increased expression of the CIO and cbb3-1 terminal oxidases. Environ Microbiol 8(10):1764-1774.

Moreno R, Martinez-Gomariz M, Yuste L, Gil C, Rojo F (2009) The Pseudomonas putida Crc global regulator controls the hierarchical assimilation of amino acids in a complete medium: Evidence from proteomic and genomic analyses. Proteomics 9:2910-2928

Nakazawa T, Yokota T (1973) Benzoate metabolism in *Pseudomonas putida* (arvilla) mt 2: Demonstration of two benzoate pathways. J Bacteriol 115:262-267

Nelson KE, Weinel C, Paulsen IT, Dodson RJ, Hilbert H, Martins dos Santos VAP, Fouts DE, Gill SR, Pop M, Holmes M and others (2002) Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440. Environ Microbiol 4:799-808

Nijkamp K, Westerhof RGM, Ballerstedt H, De Bont JAM, Wery J (2007) Optimization of the solvent-tolerant *Pseudomonas putida* S12 as host for the production of p-coumarate from glucose. Appl Microbiol Biotechnol 74:617-624

Nikodinovic-Runic J, Flanagan M, Hume AR, Cagney G, O'Connor KE (2009) Analysis of the *Pseudomonas putida* CA-3 proteome during growth on styrene under nitrogen-limiting and non-limiting conditions. Microbiol 155:3348-3361

Nogales J, Palsson BO, Thiele I (2008) A genome-scale metabolic reconstruction of *Pseudomonas putida* KT2440: iJN746 as a cell factory. BMC Syst Biol 2:79

Ouyang SP, Luo RC, Chen SS, Liu Q, Chung A, Wu Q, Chen GQ (2007) Production of polyhydroxyalkanoates with high 3-hydroxydodecanoate monomer content by *fadB* and *fadA* knockout mutant of *Pseudomonas putida* KT2442. Biomacromolecules 8:2504-2511

Patel RN, Banerjee A, Ko RY, Howell JM, Li Wen S, Comezoglu FT, Partyka RA, Szarka L (1994) Enzymic preparation of (3R-cis)-3-(acetyloxy)-4-phenyl-2-azetidinone: A taxol side-chain synthon. Biotechnol Appl Biochem 20:23-33

Puchalka J, Oberhardt MA, Godinho M, Bielecka A, Regenhardt D, Timmis KN, Papin JA, Martins dos Santos VA (2008) Genome-scale reconstruction and analysis of

the *Pseudomonas putida* KT2440 metabolic network facilitates applications in biotechnology. PLoS Comput Biol 4:e1000210

Rehm BHA (2010) Bacterial polymers: Biosynthesis, modifications and applications. Nat Rev Microbiol 8:578-592.

Renzi F, Rescalli E, Galli E, Bertoni G (2010) Identification of genes regulated by the MvaT-like paralogues TurA and TurB of *Pseudomonas putida* KT2440. Environ Microbiol 12:254-263

Reva ON, Weinel C, Weinel M, Bohm K, Stjepandic D, Hoheisel JD, Tummler B (2006) Functional genomics of stress response in *Pseudomonas putida* KT2440. J Bacteriol 188:4079-4092

Ronchel MC, Molina L, Witte A, Lutbiz W, Molin S, Ramos JL, Ramos C (1998) Characterization of cell lysis in *Pseudomonas putida* induced upon expression of heterologous killing genes. Appl Environ Microbiol 64:4904-4911

Rosche B, Li XZ, Hauer B, Schmid A, Buehler K (2009) Microbial biofilms: a concept for industrial catalysis? Trends Biotechnol 27:636-643

Rühl J, Schmid A, Blank LM (2009) Selected *Pseudomonas putida* strains able to grow in the presence of high butanol concentrations. Appl Environ Microbiol 75:4653-4656

Santos PM, Benndorf D, Sa-Correia I (2004) Insights into *Pseudomonas putida* KT2440 response to phenol-induced stress by quantitative proteomics. Proteomics 4:2640-2652

Schmid A, Dordick JS, Hauer B, Kiener A, Wubbolts M, Witholt B (2001) Industrial biocatalysis today and tomorrow. Nat 409:258-268

Schulze B, Wubbolts MG. 1999. Biocatalysis for industrial production of fine chemicals. Curr Opinion Biotechnol 10:609-615

Segura A, Godoy P, Van Dillewijn P, Hurtado A, Arroyo N, Santacruz S, Ramos JL (2005) Proteomic analysis reveals the participation of energy- and stress-related proteins in the response of *Pseudomonas putida* DOT-T1E to toluene. J Bacteriol 187:5937-5945

Silva-Rocha R, de Jong H, Tamames J, de Lorenzo V (2011) The logic layout of the TOL network of *Pseudomonas putida* pWW0 plasmid stems from a metabolic amplifier motif (MAM) that optimizes biodegradation of m-xylene. BMC Syst Biol:191.

Sohn SB, Kim TY, Park JM, Lee SY (2010) In silico genome-scale metabolic analysis of *Pseudomonas putida* KT2440 for polyhydroxyalkanoate synthesis, degradation of aromatics and anaerobic survival. Biotechnol J 5:739-750

Stephan S, Heinzle E, Wenzel SC, Krug D, Müller R, Wittmann C (2006) Metabolic physiology of *Pseudomonas putida* for heterologous production of myxochromide. Proc Biochem 41:2146-2152

Stieglitz B, Dicosimo R, Fallon RD (1996) Formation of aliphatic ω-cyanocarboxamide(s) from α,ω-dinitrile(s) – using bio-catalyst having regioselective nitrile hydratase activity derived from *Pseudomonas putida*. US Patent US 5728556

Sun Z, Ramsay J, Guay M, Ramsay B (2006) Automated feeding strategies for high-cell-density fed-batch cultivation of *Pseudomonas putida* KT2440. Appl Microbiol Biotechnol 71:423-431

Sun Z, Ramsay J, Guay M, Ramsay B (2007) Carbon-limited fed-batch production of medium-chain-length polyhydroxyalkanoates from nonanoic acid by *Pseudomonas putida* KT2440. Appl Microbiol Biotechnol 74:69-77

Sun Z, Ramsay J, Guay M, Ramsay B (2009) Fed-batch production of unsaturated medium-chain-length polyhydroxyalkanoates with controlled composition by *Pseudomonas putida* KT2440. Appl Microbiol Biotechnol 82:657-662

Tang H, Yu H, Li Q, Wang X, Gai Z, Yin G, Su F, Tao F, Ma C, Xu P (2011) Genome Sequence of *Pseudomonas putida* Strain B6-2, a Superdegrader of Polycyclic Aromatic Hydrocarbons and Dioxin-Like Compounds. J Bacteriol 193:6789-6790

Tao F, Tang H, Gai Z, Su F, Wang X, He X, Xu P. 2011. Genome Sequence of *Pseudomonas putida* Idaho, a Unique Organic-Solvent-Tolerant Bacterium. J Bacteriol 193:7011-7012

Timmis KN (2002) *Pseudomonas putida*: A cosmopolitan opportunist par excellence. Environ Microbiol 4:779-781

Tsirogianni E, Aivaliotis M, Papasotiriou DG, Karas M, Tsiotis G (2006) Identification of inducible protein complexes in the phenol degrader Pseudomonas sp. strain phDV1 by blue native gel electrophoresis and mass spectrometry. Amin Ac 30:63-72

Ütkur FO, Gaykaward S, Buehler B, Schmid A (2011) Regioselective aromatic hydroxylation of quinaldine by water using quinaldine 4-oxidase in recombinant *Pseudomonas putida*. J Ind Microb Biotechnol 38:1067-1073

Van Der Werf MJ, Overkamp KM, Muilwijk B, Koek MM, Van Der Werff-Van Der Vat BJC, Jellema RH, Coulier L, Hankemeier T (2008) Comprehensive analysis of

the metabolome of *Pseudomonas putida* S12 grown on different carbon sources. Mol BioSyst 4:315-327

Van Duuren JBJH (2011) Optimization of *Pseudomonas putida* KT2440 as host for the production of cis, cis-muconate from benzoate. Dissertation

Van Duuren JBJH, Brehmer B, Mars AE, Eggink G, dos Santos VM, Sanders JPM (2011a) A limited LCA of bio-adipic acid: Manufacturing the nylon-6,6 precursor adipic acid using the benzoic acid degradation pathway from different feedstocks. Biotechnol Bioeng 108:1298-1306

Van Duuren JBJH, Wijte D, Karge B, Martins dos Santos VA, Yang Y, Mars AE, Eggink G (2011b) pH-stat fed-batch process to enhance the production of *cis, cis*-muconate from benzoate by *Pseudomonas putida* KT2440-JD1. Biotechnol Prog

Van Duuren JBJH, Wijte D, Leprince A, Karge B, Puchalka J, Wery J, Dos Santos VAPM, Eggink G, Mars AE (2011c) Generation of a *catR* deficient mutant of *P. putida* KT2440 that produces *cis, cis*-muconate from benzoate at high rate and yield. J Biotechnol 156:163-172

Verhoef S, Ballerstedt H, Volkers RJM, De Winde JH, Ruijssenaars HJ (2010) Comparative transcriptomics and proteomics of p-hydroxybenzoate producing *Pseudomonas putida* S12: Novel responses and implications for strain improvement. Appl Microbiol Biotechnol 87:679-690

Verhoef S, Ruijssenaars HJ, de Bont JAM, Wery J (2007) Bioproduction of p-hydroxybenzoate from renewable feedstock by solvent-tolerant *Pseudomonas putida* S12. J Biotechnol 132:49-56

Verhoef S, Wierckx N, Westerhof RGM, De Winde JH, Ruijssenaars HJ (2009) Bioproduction of p-hydroxystyrene from glucose by the solvent-tolerant bacterium *Pseudomonas putida* S12 in a two-phase water-decanol fermentation. Appl Environ Microbiol 75:931-936

Volkers RJM, De Jong AL, Hulst AG, Van Baar BLM, De Bont JAM, Wery J (2006) Chemostat-based proteomic analysis of toluene-affected *Pseudomonas putida* S12. Environ Microbiol 8:1674-1679

Wang HH, Zhou XR, Liu Q, Chen GQ (2011) Biosynthesis of polyhydroxyalkanoate homopolymers by *Pseudomonas putida*. Appl Microbiol Biotechnol 89:1497-1507

Wenzel SC, Gross F, Zhang Y, Fu J, Stewart AF, Müller R (2005) Heterologous expression of a myxobacterial natural products assembly line in Pseudomonads via Red/ET recombineering. Chem Biol 12:349-356

Wierckx N, Ruijssenaars HJ, de Winde JH, Schmid A, Blank LM (2009) Metabolic flux analysis of a phenol producing mutant of *Pseudomonas putida* S12: Verification and complementation of hypotheses derived from transcriptomics. J Biotechnol 143:124-129

Wierckx NJP, Ballerstedt H, De Bont JAM, De Winde JH, Ruijssenaars HJ, Wery J (2008) Transcriptome analysis of a phenol-producing *Pseudomonas putida* S12 construct: Genetic and physiological basis for improved production. J Bacteriol 190:2822-2830

Wierckx NJP, Ballerstedt H, De Bont JAM, Wery J (2005) Engineering of solvent-tolerant *Pseudomonas putida* S12 for bioproduction of phenol from glucose. Appl Environ Microbiol 71:8221-8227

Wolff JA, MacGregor CH, Eisenberg RC, Phibbs Jr PV (1991) Isolation and characterization of catabolite repression control mutants of *pseudomonas aeruginosa* pao. J Bacteriol 173:4700-4706

Wong JW, Watson HA, Bouressa JF, Burns MP, Cawley JJ, Doro AE, Guzek DB, Hintz MA, McCormick EL, Scully DA and others (2002) Biocatalytic Oxidation of 2-Methylquinoxaline to 2-Quinoxalinecarboxylic Acid. Org Proc Res Devel 6:477-481

Yang T, Jung Y, Kang H, Kim T, Park S, Lee S (2011) Tailor-made type II *Pseudomonas* PHA synthases and their use for the biosynthesis of polylactic acid and its copolymer in recombinant *Escherichia coli*. Appl Microbiol Biotechnol 90:603-614

Yeom S, Yeom J, Park W (2010) NtrC-sensed nitrogen availability is important for oxidative stress defense in *Pseudomonas putida* KT2440. J Microbiol 48:153-159

Yu H, Tang H, Wang L, Yao Y, Wu G, Xu P (2011) Complete genome sequence of the nicotine-degrading *Pseudomonas putida* strain S16. J Bacteriol 193:5541-5542

Zhen D, Liu H, Wang SJ, Zhang JJ, Zhao F, Zhou NY (2006) Plasmid-mediated degradation of 4-chloronitrobenzene by newly isolated *Pseudomonas putida* strain ZWL73. Appl Microbiol Biotechnol 72:797-803

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

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)

[a] Batch culture

[b] Chemostat culture

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

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)

1Table 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 (*phaC*), plasmid-based expression of the PHA synthesis operon (*phaPCJ*) from Aeromonas hydrophila; valerate used as carbon source	Wang 2011
PHA C_3 and C_4 homo-polymer	KTHH03	KTHH06	Replacement of endogenous *phaC* by *phaC* from *Ralstonia eutropha*; γ-butyrolactone as precursor	Wang 2011
Myxochromide S	KT2440	P.putida::CMch37a	Heterologous expression of myxochromide S biosynthetic gene cluster from *Stigmatella aurantiaca*	Stephan 2006
--	KT2440	FG2005	Integration of methyl-malonyl-CoA pathway from *Sorangium cellulosum*	Gross 2006
Myxothiazol	FG2005	FG2005::Pm-mta	Integration of myxothiazol synthesis cluster from *Stigmatella aurantiaca*	Gross 2006
p-Hydroxysterene	S12	S12 427Δfcs pJNTpalpdc	Introduction of *pal* (*R. glutinis*) and *pdc* (*L. plantarum*), inactivating *fcs* genes	Verhoef 2009
D-Glucosaminic acid	GNA5	GNA5	D-glucosaminic acid accumulated during oxidative fermentation process	Wu 2010
Phenol	S12	S12	Introduction of *tpl* gene from *Pantea agglomeran*, overexpression of the *aroF-1* gene	Wierckx 2005
p-Coumarate	S12	S12 C3	Inactivation of fcs, construction of phenylalanine auxothrophic 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 *todC1C2BAD* under control of an inducible regulatory region	Hüsken 2001
o-Cresol	T-57	TODD1	*todD* knockout mutant of T-57	Faizal 2005
Aliphatic alcohols	PpS81	PpS8141	Introduction of the *alk* regulon (*alkBFGH/alkST*) from *Pseudomonas oleovorany*	Bosetti 1992
4-Valerolactone	KT2440	KT2440	Introduction of *tesB* (*E. coli* G1655) to secrete 4-hydroxyvalerate and pon1 (human paraoxonase I), expressed extra-cytosolic to catalyze the	Martin 2010

			utilization the intermediate 4-hydroxyvalerate	
p-Hydroxybenzoate	S12	S12pal_xylB7	Additional copy of *aroF-1* gene, introduction of *xylAB_FGH* genes (*E.coli*) and the *pal* gene (*R. toruloides*)	Meijnen 2011
2-Alkyl-4(1H)-quinolones and related derivates	KT2440	KT2440 [pBBR-pqsABCD]	Insertion of *pqsABCD* from *P. aeruginosa*	Niewerth 2011
Styreneoxide	DOT-T1E	DOT-T1E [pTEZ240]	Heterologous expression of styrene monooxygenase StyAB from *P. spec*. VLB120	Blank 2008
4-Hydroxyquinaldine	KT2440	KT2440 (pKP1)	Inserted genes *qoxLMS* (*Arthrobacter nitroguajoccolicus* Rü61a)	Ütkür 2011
Biodesulfurization	S12	DS23	Insertion of desulfurizing gene cluster (*dszABCD)* (*P. putida* A4)	Tao 2011
Bioconversion of limonin	G7	G7	Use of whole cells permeabilized with EDTA and lysozyme	Malik 2011

Table 4. Industrial processes based on *Pseudomonas* strains

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

Figure Legends

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

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