Biotechnology Journal
www.biotechnology-journal.com

Pseudomonas as Versatile Aromatics Cell Factory

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Aromatics and their derivatives are valuable chemicals with a plethora of important applications and thus play an integral role in modern society. Their current production relies mostly on the exploitation of petroleum resources. Independency from dwindling fossil resources and rising environmental concerns are major driving forces for the transition towards the production of sustainable aromatics from renewable feedstocks or waste streams.

Whole-cell biocatalysis is a promising strategy that allows the valorization of highly abundant, low-cost substrates. In the last decades, extensive efforts are undertaken to allow the production of a wide spectrum of different aromatics and derivatives using microbes as biocatalysts. Pseudomonads are intriguing hosts for biocatalysis, as they display unique characteristics beneficial for the production of aromatics, including a distinct tolerance and versatile metabolism.

This review highlights biotechnological applications of *Pseudomonas* as host for the production of aromatics and derived compounds. This includes their *de novo* biosynthesis from renewable resources, biotransformations in single- and biphasic fermentation setups, metabolic funneling of lignin-derived aromatics, and the upcycling of aromatic monomers from plastic waste streams. Additionally, this review provides insights into unique features of Pseudomonads that make them exceptional hosts for aromatics biotechnology and discusses engineering strategies.

1. Introduction

Aromatic and aromatics-derived compounds (ADC) are valuable bulk or fine chemicals with a myriad of important applications. They serve as building blocks for many valuable compounds including polymers such as plastics, resins, and fibers and are used as lubricants, dyes, and pesticides. Moreover, they are needed for the production of indispensable nutra- and pharmaceuticals.[1–4] Their vast majority is produced from petroleum in energy-intensive and environment-polluting processes. The increasing demand, the limitation of fossil resources, and the current environmental crisis have drawn rising attention to the much-required shift toward a sustainable, bio-based production of fuels and chemicals from renewable feedstocks.[5] Next to green chemistry, microbial biocatalysis is a promising strategy to produce aromatics at ambient temperatures and pressure without the use of toxic catalysts, thereby reducing energy consumption and waste generation.[6] The progress in this field has been recently reviewed in multiple publications giving a broad overview for several host organisms.[1–4,7,8] The here presented review specifically highlights the application of different *Pseudomonas* species as microbial cell factory for the production of industrially relevant aromatics and ADC. This includes the de novo synthesis of such from renewable and abundantly available feedstocks and associated genetic engineering strategies enabling efficient bioconversion. Moreover, biotransformation approaches of natural and fabricated aromatic substrates into value-added products are elucidated, with a focus on the unique stress resistance of *Pseudomonas* enabling the use of organic solvents in biphasic fermentations. The valorization of lignin- and plastic-derivable aromatics and metabolic funneling of heterogeneous mixtures thereof is also discussed.

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The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/biot.201900569

DOI: 10.1002/biot.201900569

2. Bioconversion

2.1. Strategies to Increase de novo Biosynthesis of Shikimate Pathway-Derived Compounds

2.1.1. Engineering of the Shikimate Pathway and Associated Anabolic and Catabolic Routes

Due to their distinct tolerance toward toxic chemicals and other stresses, pseudomonads have become popular workhorses for the de novo synthesis of aromatics and ADC. An extensive overview of products and applied pathways can be found in **Figure** 1 and **Table 1**.

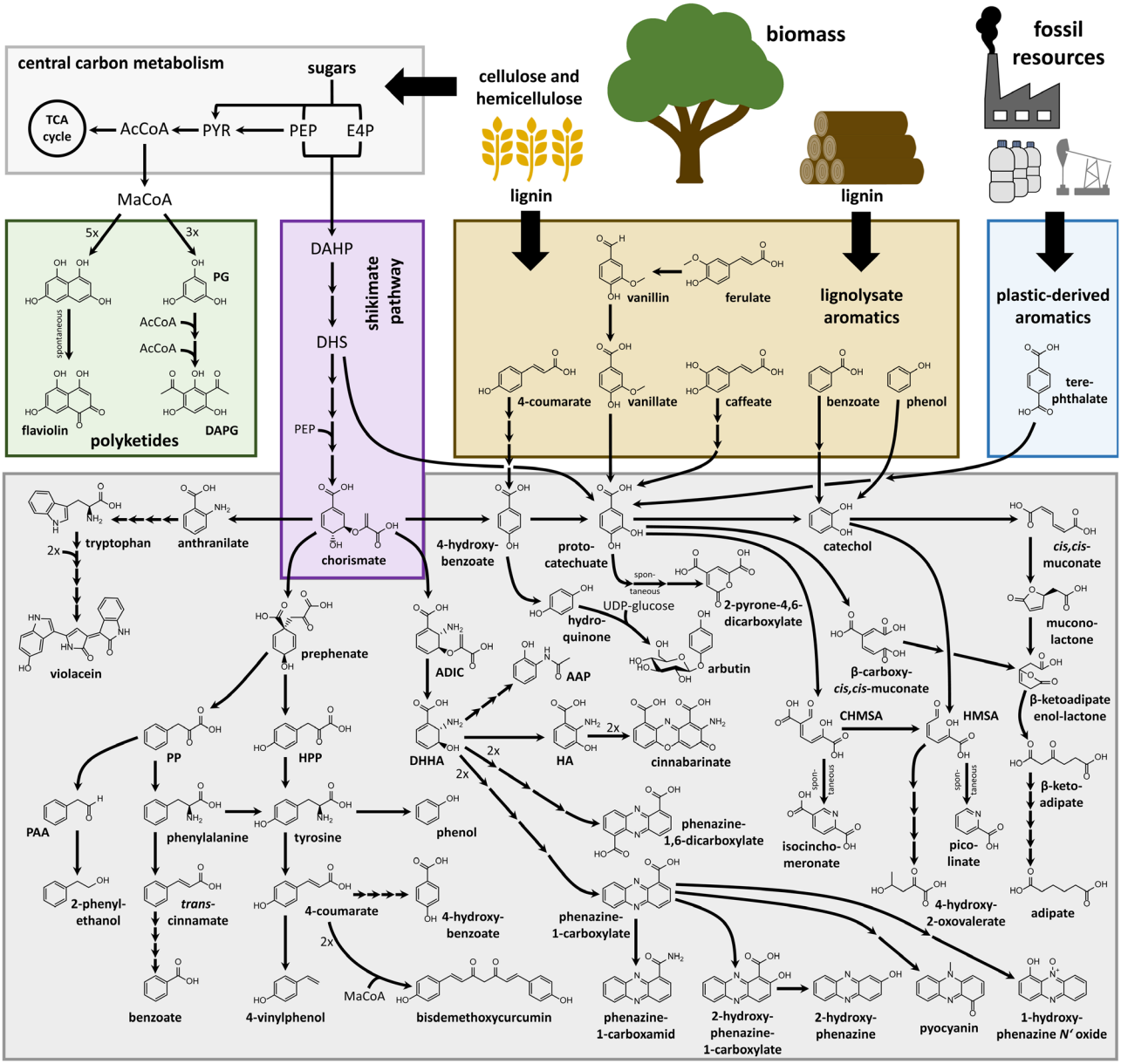

Figure 1. Schematic overview of pathways applied, and compounds produced with *Pseudomonas*. Illustrated are aromatics and derived compounds that were produced using *Pseudomonas*. This includes their de novo production and biotransformation approaches using aromatic monomers derivable from lignin or anthropogenic polymers. Arrows indicate reactions that can be catalyzed by native or heterologous enzymes. Abbreviations: AAP, 2-acetamidophenol; E4P, erythrose 4-phosphate; PEP, phosphoenolpyruvate; PYR, pyruvate; AcCoA, acetyl-CoA; MaCoA, malonyl-CoA; PG, phloroglucinol; DAPG, 2,4-diacetylphloroglucinol; DAHP, 3-deoxy-D-arabinoheptulosonate 7-phosphate; DHS, 3-dehydroshikimate; PP, phenylpyruvate; HPP, 4-hydroxyphenylpyruvate; PAA, phenylacetaldehyde; ADIC, 2-amino-2-deoxyisochorismate; DHHA, *trans*-2,3-dihydro-3-hydroxyanthranilate; HA, 3-hydroxyanthranilate; CHMSA, 5-carboxy-2-hydroxymuconate-semialdehyde; HMSA, 2-hydroxymuconate-semialdehyde.

Initial studies predominately applied mutagenesis and screening to enhance product formation, due to knowledge limitations at this time in comparison to more traditional hosts like *Escherichia coli*. In fact, initial attempts to overproduce aromatics by overexpression of typical *E. coli* bottleneck genes only yielded moderate results, indicating that *Pseudomonas*' versatile metabolism includes a unique architecture and regulation around the shikimate pathway.[9,10] In *P. putida* S12 the synthesis of *trans*-cinnamate,[11] phenol,[9] and 4-coumarate[12] was improved by chemical mutagenesis and antimetabolite selection followed by high-throughput screening (>10000 mutants). Through these efforts, overproducers were obtained that achieved yields of 3.2, 6.7, and 6.5% Cmol Cmol^{-1} for *trans*-cinnamate, phenol, and 4-coumarate, respectively, from glucose in simple shake flasks experiments.[9,11,12] This strategy is labor-intensive, yet powerful, and mutants can be obtained that harbor modifications in so far unknown targets. Due to this benefit, a more recent study pursued a similar workflow that

enabled enhanced production of phenylalanine in *P. putida* DOT-T1E followed by targeted metabolic engineering.[13] Characterization of such mutants is a key factor to shed light into the mechanisms of enhanced aromatics biosynthesis and to identify targets for rational engineering. This can be achieved by systems biology approaches such as transcriptomics, proteomics, metabolomics, and genome re-sequencing.[14–17] Recently, identified leads from phenol-producing *P. putida* S12TPL3 and *trans*-cinnamate-producing S12palM12 were reverse-engineered in *P. taiwanensis* VLB120 to study the isolated and combined effects of several modifications on phenol production via tyrosine.[10] This approach, coupled to forward engineering of commonly known targets, enabled enhanced yields of 15.6 and 22.7% Cmol Cmol^{-1} on glucose and glycerol, respectively.[10,18]

The ever-increasing wealth of available synthetic biology tools and the gain of knowledge allow considerably faster and more efficient engineering of *Pseudomonas* that is more and more knowledge-based. The deletion of aromatics catabolic pathways is an important aspect in this. The much-heralded metabolic versatility of pseudomonads[19–21] is a double-edged blade. Their degradative power is often a benefit in transformation and funneling approaches.[22] However, the degradation of a multitude of aromatic compounds and their precursors can also hinder de novo aromatics production. It was shown that aromatics-degrading pathways were upregulated as a response to the enhanced flux into the shikimate pathway,[14,16] indicating a potential futile cycle of anabolism and catabolism which limits product formation. Indeed, subsequent elimination of the tyrosine-metabolizing homogentisate pathway in *P. putida* S12palB1, that was derived from mutagenized phenol-producing S12TPL3, further enhanced 4-hydroxybenzoate production.[16,23] Therefore, current studies usually begin with the inactivation of undesired aromatics-catabolizing pathways.[10,24–26]

However, the elimination of product- or precursor-metabolizing pathways per se is not expected to show large effects on product formation without a deregulated flux into and through the shikimate pathway. In order to increase this flux, known bottlenecks must be eliminated. The first committed and rate-limiting reaction of the shikimate pathway is catalyzed by 3-deoxy-D-arabinoheptulosonate 7-phosphate (DAHP) synthase that condenses phosphoenolpyruvate (PEP) and erythrose 4-phosphate (E4P) to DAHP.[27] Respective isozymes are usually subject to allosteric inhibition of biosynthetic end products of the primary metabolism such as tyrosine and tryptophan.[28] To circumvent this, feedback resistant (fbr) variants of DAHP synthases were overexpressed—in many cases AroG^{fbr} derived from *E. coli*.[10,25,26,29–31] However, *P. aeruginosa* and *P. chlororaphis* possess an additional DAHP synthase encoded by the secondary metabolite gene cluster for the production of phenazines. This DAHP synthase, PhzC, is allosterically insensitive to aromatic amino acids and phenazines[32] and thus also a suitable overexpression candidate.[33,34]

The branching point at the level of chorismate, the end product of the shikimate pathway, is another tightly regulated node since at this point the fate of the metabolic flux to either tryptophan, phenylalanine, tyrosine, ubiquinone, folate, or secondary metabolites is determined. The regulation can be mediated on a transcriptional, translational, and/or allosteric level.[35–37] Like for DAHP synthase, initial branch-specific feedback-resistant enzyme variants are often overexpressed, if available, to enhance the flux toward the target product. The branch-specific enzymes are the anthranilate synthase (TrpE^{fbr}G),[30] bifunctional chorismate mutase with either prephenate dehydratase (PheA^{fbr})[13,25] or dehydrogenase activity (TyrA^{fbr}),[10,29] and chorismate pyruvate-lyase (UbiC/UbiC^{fbr}/XanB2)[26,38,39] for the tryptophan, phenylalanine, tyrosine, and 4-hydroxybenzoate branch, respectively. Instead of introducing heterologous genes, also inherent genes can be genetically targeted in their native locus to achieve feedback insensitivity.[10] This has the major benefit that it avoids the high metabolic burden caused by overexpression that can lead to impaired growth and a reduced reproducibility.[25] Although, PheA^{fbr} increases the flux toward phenylalanine, this enzyme can also be overexpressed in the context of tyrosine overproduction, because *Pseudomonas*, unlike *E. coli*, can metabolize phenylalanine to tyrosine.[20] In fact, in tyrosine-overproducing strains, the majority of the metabolic flux toward the product is channeled via phenylalanine.[14,25]

In addition to the overexpression of the initial branch enzyme, chorismate-competing pathways can be eliminated or down-regulated. The elimination of pathways for secondary metabolites derived from chorismate usually does not impair growth,[33] while those of essential building blocks or vitamins result in auxotrophies.[26,30] The addition of supplements under limiting conditions can be exploited to alleviate end product inhibition or repression to enhance product formation as demonstrated by Küpper et al.[30] Here, a tryptophan-limited fed-batch fermentation of a tryptophan-auxotrophic strain was employed to produce anthranilate. Yu et al.[26] established efficient 4-hydroxybenzoate production in *P. putida* KT2440 via the chorismate pyruvate-lyase pathway. The applied pathway features a higher carbon-efficiency than the 4-hydroxybenzoate route via tyrosine. However, the deletion of *trpE* and *pheA* yielded a tryptophan- and phenylalanine/tyrosine-auxotrophic strain. This necessitates supplementation of these aromatic amino acids or their precursors. Intriguingly, in *Pseudomonas* the role and/or regulation of PheA seems to be different than in *E. coli*. It was reported that it is hard to knock out and that this deletion leads to a growth deficiency[26,30] that could only be fully reverted by the supplementation of high phenylpyruvate concentrations.[30] Regarding the production of anthranilate, the best *P. putida* KT2440 strain still possessed *pheA* and outperformed its *pheA*-deficient counterpart,[30] which was associated to a reduced fitness mediated by Δ*pheA*. Although the generation of auxotrophic strains is a popular strategy to prevent product loss to precursor-competing molecules,[40,41] the addition of essential aromatics, such as tryptophan, phenylalanine, and tyrosine is a major cost factor that is often not considered. Instead of deleting competing pathways of essential metabolites, it can be favorable to only decrease the flux and allow their limited production. This so-called bradytrophy can be achieved by tuning gene expression, for example, by replacing the native promoter, ribosome-binding site or start codon or by altering enzymatic activities. In chorismate-derived secondary metabolite-producing *P. aeruginosa* the chorismate pyruvate-lyase UbiC was replaced by one from *Mycobacterium tuberculosis* with a reduced enzymatic activity.[33] This led to reduced carbon loss to the 4-hydroxybenzoate branch, while the native PheA was modified (amino acid substitution: W323L) to decrease the flux toward phenylalanine and tyrosine.[33] In

mutagenized, phenol-producing *P. putida* S12TPL3 a mutation in *trpE* was identified resulting in a P290S amino acid substitution in anthranilate synthase subunit I. The respective strain was tryptophan-bradytrophic, indicating a reduced activity of the respective enzyme. In reverse-engineered *P. taiwanensis* VLB120, this mutation was foundational to enhance phenol, 4-hydroxybenzoate, *trans*-cinnamate, and benzoate production.[10,25,29,42] The resulting enhanced biosynthesis cannot only be explained by a reduced carbon loss to the competing tryptophan biosynthesis pathway alone. Tryptophan-limited growth conditions likely led to a cellular response leading to a transcriptional upregulation of the shikimate pathway and reduced allosteric inhibition, thus resulting in an elevated flux.[10,14] Mutations like this can be beneficial for product formation, while still allowing the biosynthesis of competing essential metabolites thereby avoiding the addition of expensive supplements.

2.1.2. Increasing the Phosphoenolpyruvate Supply

To further enhance the flux into the shikimate pathway, the supply of the central metabolites PEP and E4P must be increased. PEP supply is usually elevated by the deletion of glycolytic reactions around the PEP node. Common deletion targets are the pyruvate (PYR) kinase (PykA, PykF)[18,31,34] and sometimes PEP carboxylase (Ppc).[18,31] Unlike *E. coli*, *Pseudomonas* does not rely on a PEP-dependent phosphotransferase system for glucose uptake. However, this advantage is negated by the fact that the primary metabolic flux mainly occurs through the Entner Doudoroff (ED) pathway,[43] which yields one glyceraldehyde 3-phosphate and one PYR per glucose molecule. The cyclic nature of the so-called EDEMP pathway that involves gluconeogenesis from glyceraldehyde 3-phosphate amplifies this effect.[44,45] However, an inactivation of the ED pathway results in mutants that are unable to grow on glucose due to an incomplete Embden-Meyerhof-Parnas pathway and a naturally low flux through the pentose phosphate pathway (PPP).[46] Recently linear glycolysis was established in a *P. putida* KT2440 ED pathway-deficient mutant.[46] The implementation of linear glycolysis could be beneficial for aromatics production to increase PEP supply from glucose. Alternatively, glycerol is an interesting carbon source with a high potential for aromatics production. It is a major byproduct of biodiesel production and thus abundantly available. Glycerol enters the central carbon metabolism at the level of dihydroxyacetone phosphate, which can be channeled directly to PEP. Glycerol is partly metabolized via the ED pathway, but the flux through this pathway is reduced.[47] Likely, this is the main reason why aromatics production on glycerol is often more efficient than on glucose in *Pseudomonas*, especially in strains where PYR kinase activity was reduced or eliminated.[10,23,25,29,48]

Although glycerol is an attractive carbon source for the production of aromatics, its abundance and pricing is heavily depending on the biodiesel market. For this reason, efficient aromatics production from glucose is desirable. A recently published strategy to disconnect the cyclic nature of the EDEMP pathway was pursued by Johnson et al.[31] to increase product formation. In addition to Δ*pykA*, Δ*pykF*, and Δ*ppc*, glucose 6-phosphate isomerase-encoding *pgi-1* and *pgi-2* were deleted to prevent gluconeogenesis from glyceraldehyde 3-phosphate. This results in an estimated carbon split of 50% being channeled into the tricarboxylic acid (TCA) cycle via PYR and 50% being available for aromatic precursors (PEP and E4P) and thus for product formation. A heterologous *cis,cis*-muconate pathway based on 3-dehydroshikimate dehydration, protocatechuate decarboxylation, and catechol conversion to *cis,cis*-muconate was implemented in this strain. Metabolic flux to the product was enhanced by overexpression of $AroG^{fbr}$ and elimination of degradation pathways of the product and its precursors. The resulting strain CJ422 achieved remarkable *cis,cis*-muconate yields from glucose of up to 33.3% (Cmol $Cmol^{-1}$) in shake flask cultivations. To simplify bioreactor fermentations glucose dehydrogenase *gcd* was deleted yielding CJ522, which also further enhanced yield and titer. In a fed-batch fermentation, a titer of 84.4 mM with a yield of 37.8% (Cmol $Cmol^{-1}$) was reached. However, growth and productivity were drastically reduced compared to CJ442.[31,49] Growth was enhanced in an evolutionary approach followed by the application of a *cis,cis*-muconate-responsive biosensor to allow high-throughput fluorescence-activated cell sorting for mutants that showed sufficient *cis,cis*-muconate production. Analysis of resulting mutants indicated several central metabolic regulators including HexR, GntZ, and GacS, whose deletion further increased strain growth and *cis,cis*-muconate production performance.[49] In a fed-batch fermentation, the best strain GB271 (= CJ522Δ*hexR*Δ*gntZ*Δ*gacS*) outperformed its progenitor CJ522 regarding *cis,cis*-muconate titer and productivity with 155 mM and 1.48 mM, h^{-1} compared to 49 mM and 0.49 mM h^{-1}, respectively, while still showing a similar yield with 35.6% Cmol $Cmol^{-1}$.[49]

2.1.3. Increasing the Erythrose 4-Phosphate Supply

Next to elevating PEP availability, the metabolic flux toward E4P also needs to be increased, which is challenging in *Pseudomonas* due to the low PPP flux in this organism. The transketolase TktA is a commonly overexpressed enzyme to increase E4P supply. In some cases, this was also successfully done in *Pseudomonas*.[34,50] However, in 4-hydroxybenzoate-producing *P. taiwanensis*, TktA expression did not increase production.[29] In 4-hydroxybenzoate-producing *P. putida* KT2440, Δ*hexR* led to enhanced production likely associated to an increased E4P supply, but only when $AroG^{fbr}$ was simultaneously overexpressed.[26]

In addition to redirecting the flux from glucose to E4P, cofeeding strategies of substrates that favor the formation of E4P can be applied.[48] Because E4P is an intermediate of the PPP, phosphorylative pentose assimilation is a promising strategy. Especially xylose and arabinose are interesting pentose sugars due to their abundance in lignocellulose.[51–53] However, pseudomonads are often naturally unable to metabolize pentoses such as xylose. Although *P. taiwanensis* VLB120 is an exception to this, this strain uses the oxidative Weimberg pathway that channels xylose into the TCA cycle.[54] Via this pathway, phenol and 4-hydroxybenzoate production from xylose as sole carbon source was demonstrated.[10,29] However, production was significantly lower compared to when glucose or glycerol were used as carbon source and phosphorylative xylose assimilation is likely more favorable for aromatics synthesis.[55] The implementation of a non-oxidative xylose catabolic pathway via the PPP enabled

Table 1. Bioconversions applying *Pseudomonas* as biocatalyst for the biosynthesis of aromatics and derived compounds.

Product	Host	Strain	Genotype, description	Substrate	Cultivation mode	Additives	Titer [mM]	Yield % [Cmol Cmol^{-1}]	Ref.
trans-cinnamate	*P. putida* S12	S12palM12	NTG mutagenesis, antimetabolite selection, high-throughput screening, *Rt*PAL expression; detected mutations: *pheA*^{T310I}, RPPX_RS16300^{T137I}	Glucose	Shake flask, batch	-	0.42	3.2	[11]
				Glucose	Bioreactor, fed-batch	Yeast extract	5.0	4.6	
				Glycerol	Bioreactor, fed-batch	Yeast extract	5.4	6.7	
	P. putida DOT-T1E	CM12-5 pPAL1	antimetabolite selection, EMS mutagenesis; ΔphhA ΔkatG Δhpd ΔquiC1 ΔpeaE, NpPAL expression	Glucose	Shake flask, batch	-	1.35	n.d.	[13]
	P. taiwanensis VLB120	GRC3Δ8Δ*pykA-tap attTn7*::P_{14g}-*At*PAL	Genome-reduced chassis, ΔpobA Δhpd ΔquiC ΔquiC1 ΔquiC2 ΔphhAB ΔkatG ΔPVLB_10 925 trpE^{P290S} aroF-1^{P148L} pheA^{T310I} ΔpykA, AtPAL expression	Glucose	Shake flask, batch	-	3.34	22.8	[25]
				Glycerol	Shake flask, batch	-	5.25	38.9	
				Glucose	Bioreactor, fed-batch	-	33.2	21.4	
				Glycerol	Bioreactor, fed-batch	-	33.5	36.1	
	P. taiwanensis VLB120	GRC3Δ8Δ*pykA-tap attTn7*::P_{14g}-*At*PAL-*aroG*^{fbr}-*pheA*^{fbr}	GRC3Δ8Δ*pykA-tap-attTn7*::P_{14g}-*At*PAL with additional overexpression of *aroG*^{fbr} and *pheA*^{fbr}	Glucose	Shake flask, batch	-	3.80	25.9	
				Glycerol	Shake flask, batch	-	6.33	47.5	
				Glucose	Bioreactor, fed-batch	-	17.2	11.2	
				Glycerol	Bioreactor, fed-batch	-	19.8	47.8	
2-phenylethanol	*P. putida* DOT-T1E	CM12-5 pPE1	antimetabolite selection, EMS mutagenesis; Δ*phhA* Δ*katG* Δ*hpd* Δ*quiC1* Δ*peaE*, expression of *hisC* (PP_0967), *Rr*KDC, and *adh* (T1E_5478)	Glucose	Shake flask, batch	-	1.47	n.d.	[13]
phenylalanine	*P. putida* DOT-T1E	CM12-5 pPHE1	antimetabolite selection, EMS mutagenesis; Δ*phhA* Δ*katG* Δ*hpd* Δ*quiC1* Δ*peaE*, expression of *pheA*^{fbr}	Glucose	Shake flask, batch	-	5.27	9.5	[13]
	P. taiwanensis VLB120	GRC3Δ8Δ*pykA-tap attTn7*::P_{14d}-*aroG*^{fbr}-*pheA*^{T310I}	genome-reduced chassis, Δ*pobA* Δ*hpd* Δ*quiC* Δ*quiC1* Δ*quiC2* Δ*phhAB* Δ*katG* ΔPVLB_10 925 *trpE*^{P290S} *aroF-1*^{P148L} *pheA*^{T310I} Δ*pykA*	Glucose	Shake flask, batch	-	3.0	22.5	[25]
tyrosine	*P. taiwanensis* VLB120	GRC3Δ5Δ*pykA-tap attTn7*::P_{14d}-*aroG*^{fbr}-*pheA*^{T310I}	genome-reduced chassis, Δ*pobA* Δ*hpd* Δ*quiC* Δ*quiC1* Δ*quiC2 trpE*^{P290S} *aroF-1*^{P148L} *pheA*^{T310I} Δ*pykA*	Glucose	Shake flask, batch	-	3.50	26.3	[25]
phenol	*P. putida* S12	S12TPL3	*Tn*(*aroF-1*) mutagenesis, NTG mutagenesis, antimetabolite selection, high-throughput screening, *Pa*TPL expression; detected mutations: *oprB-1*::*Tn*(*aroF-1*), *aroF-2*^{G136E}, *quiC1*^{P62S}, *trpE*^{P290S}, *pykA*^{V219A}, *srpB*^{R594Q}	Glucose	Shake flask, batch	-	1.48	6.7	[9]
				Glucose	Bioreactor, fed-batch	Yeast extract	5.0	2.8	
				Glucose	Bioreactor, biphasic fed-batch	Yeast extract, *n*-octanol	9.2	n.d.	
	P. taiwanensis VLB120	VLB120 Δ5-TPL36	Δ*pobA* Δ*hpd* Δ*quiC* Δ*quiC1* Δ*quiC2* Δ*pykA* pSTY^{-} *trpE*^{P290S} *aroF-1*^{P148L} *pheA*^{T310I}, expression of *Pa*TPL, *aroG*^{fbr} and *tyrA*^{fbr}	Glucose	Shake flask, batch	-	3.12	15.8	[10]
				Glycerol	Shake flask, batch	-	3.62	18.5	
				Xylose	Shake flask, batch	-	1.43	7.1	
	P. taiwanensis VLB120	VLB120 Δ5-TPL38	Δ5-TPL36 [10] with Δ*ppc*	Glycerol	Shake flask, batch	-	4.46	22.7	[18]

(*Continued*)

Table 1. Continued.

Product	Host	Strain	Genotype, description	Substrate	Cultivation mode	Additives	Titer [mM]	Yield % [Cmol Cmol^{-1}]	Ref.
	P. taiwanensis VLB120	GRC1 Δ5-TPL38	genome-reduced chassis of VLB120Δ5-TPL38	Glycerol	Shake flask, batch	-	4.74	24.1	
4-coumarate	*P. putida* S12	C3	NTG mutagenesis, antimetabolite selection, *fcs*::*tetA*, high-throughput screening; *Rt*PAL expression	Glucose Glucose	Shake flask, batch Bioreactor, fed-batch	Phenylalanine Phenylalanine	0.86 10.6	6.5 3.8	[12]
	P. putida KT2440	Δ*fcs*Δ*hpd* pPCV343 pPCV203	Δ*fcs* Δ*hpd* expression of *Fj*TAL, *aroG*^{fbr} and *tyrA*^{fbr}	Glucose	Shake flask, batch	-	1.20	6.5	[24]
4-hydroxybenzoate	*P. putida* S12	S12palB1	derived from TPL3,[9] cured from TPL-expression plasmid, *pobA*::*tetA*, *Rt*PAL expression	Glucose Glycerol Glycerol	Shake flask, batch Shake flask, batch Bioreactor, fed-batch	- - -	1.72 1.85 12.9	10.7 11.0 8.50	[23]
	P. putida S12	S12palB2	derived from TPL3,[9] cured from TPL-expression plasmid, Δ*pobA* Δ*hpd Rt*PAL expression	Glucose	Shake flask, batch	-	2.3	13.4	[16]
	P. putida S12	S12pal_xylB7	derived from palB2 [16] with Δ*gcd*, expression of xyAB_FGH from *E. coli*, evolutionary selection on xylose for enhanced growth, *Rt*PAL expression	Glucose Glycerol Xylose	Shake flask, batch Shake flask, batch Shake flask, batch	- - -	1.49 1.65 1.06	17.4 19.3 12.4	[48]
	P. putida S12	S12palB5	derived from TPL3,[9] cured from TPL-expression plasmid, Δ*pobA* Δ*hpd Rt*PAL expression	Glycerol Crude glycerol	Bioreactor, fed-batch Bioreactor, fed-batch	- 3% (w/v) non-glycerol carbon	38.4 43.4	5.9 6.6 (6.1)[a]	[223]
	P. putida KT2440	S6	Δ*pobA* Δ*trpE* Δ*pheA* Δ*hexR*, expression of *ubiC* and *aroG*^{fbr}	Glucose Glucose	Shake flask, batch Bioreactor, fed-batch	Tryptophan, phenylpyruvate Tryptophan, phenylpyruvate	7.0 18.1	4.96 12.5	[26]
	P. taiwanensis VLB120	CL4.3	Δ*pobA* Δ*hpd* Δ*quiC* Δ*quiC1* Δ*quiC2* pSTY^{-} *trpE*^{P290S}, expression of *fcs*, *ech*, *vdh*, *Rs*TAL, *aroG*^{fbr}, *tyrA*^{fbr}, *ppsA* and *pgi*	Glucose	Shake flask, batch	-	3.26	19.0	[29]
	P. taiwanensis VLB120	CL.3	Δ*pobA* Δ*hpd* Δ*quiC* Δ*quiC1* Δ*quiC2* pSTY^{-} *trpE*^{P290S}, expression of *fcs*, *ech*, *vdh*, *Rt*PAL, *aroG*^{fbr}, *tyrA*^{fbr}, *ppsA* and *pgi*	Xylose	Shake flask, batch	-	1.37	8.0	
	P. taiwanensis VLB120	CL3.3	derived from CL3 additionally overexpressing *ppsA* and *pgi*	Glycerol Glycerol	Shake flask, batch Bioreactor, fed-batch	- -	5.01 72.0	29.6 19.2	
4-vinylphenol	*P. putida* S12	427Δ*fcs* pJNT*palpdc*	derived from TPL3,[9] cured from TPL-expression plasmid, *fcs*::*tetA*, *Rt*PAL and *Lp*PDC expression	Glucose Glucose Glucose	Shake flask, batch Bioreactor, fed-batch Bioreactor, biphasic fed-batch	- - *n*-decanol	1.10 4.50 21.0	7.4 6.7 4.1	[57]
anthranilate	*P. putida* KT2440	ΔtrpDC pSEVA234_*aroG*^{D146N}_*trpE*^{S40F}*G*	Δ*trpDC*, expression of *trpE*^{fbr}, *trpG*, and *aroG*^{fbr}	Glucose Glucose	Shake flask, batch Bioreactor, fed-batch	Tryptophan Tryptophan	1.83 11.2	n.d. 5.4	[30]
arbutin	*P. chlororaphis* HT66	P3-Ar5	derived from strain P3, with Δ*pobA* Δ*rpeA* Δ*rsmE* Δ*lon* Δ*phzE* Δ*pykA* expressing *xanB2*, *mnx1*, *as*, *phzC*	Glycerol Glycerol Glucose	Shake flask, batch Shake flask, batch	20 g L^{-1} tryptone 20 g L^{-1} soy peptone, 4 g L^{-1} Tween-80	8.15 16.0	16.2[b] n.d.[b]	[39]

(*Continued*)

Table 1. Continued.

Product	Host	Strain	Genotype, description	Substrate	Cultivation mode	Additives	Titer [mM]	Yield % [Cmol Cmol^{-1}]	Ref.
benzoate	*P. taiwanensis* VLB120	GRC3Δ8Δ*pykA-tap*Δ*benABCD attTn7::P$_{14f}$-phdBCDE-4cl-pal*	genome-reduced chassis, Δ*pobA* Δ*hpd* Δ*quiC* Δ*quiC1* Δ*quiC2* Δ*phhAB* Δ*katG* Δ*PVLB_10 925* *trpE^{P290S} aroF-1^{P148L} pheA^{T310I}* Δ*pykA*, Δ*benABCD attTn7::P$_{14f}$-phdBCDE-4cl-pal attTn7::P$_{14g}$-phdBCDE- phdBCDE-4cl-pal*	Glucose	Shake flask, batch	-	1.87	10.8	[42]
				Glycerol	Shake flask, batch	-	2.97	17.3	
catechol	*P. taiwanensis* VLB120	GRC3Δ8Δ*pykA-tap*Δ*catBCD attTn7::P$_{14f}$-phdBCDE-4cl-pal*	genome-reduced chassis, ΔpobA Δhpd ΔquiC ΔquiC1 ΔquiC2 ΔphhAB ΔkatG ΔPVLB_10 925 trpE^{P290S} aroF-1^{P148L} pheA^{T310I} ΔpykA, ΔcatBCD attTn7::P14f-phdBCDE-4cl-pal	Glucose	Shake flask, batch	-	0.43	2.2	[42]
				Glycerol	Shake flask, batch	-	0.67	3.3	
cis,cis-muconate	*P. putida* KT2440	CJ200	Δ*catRBC*::P$_{tac}$-*catA*	Glucose	Shake flask, batch	-	2.3	4.7	[206]
			Δ*pcaHG*::*P$_{tac}$-aroY-ecdB-asbF*	Glucose	Bioreactor, fed-batch	-	34.6	7.7	
	P. putida KT2440	NP015	Δ*catRBC*::P$_{tac}$-*catA* Δ*pcaHG*::*P$_{tac}$-aroY-ecdB-asbF-pobR-DM-ubiC-22*	Glucose	Shake flask, batch	-	4.3	8.6	[38]
	P. putida KT2440	CJ422	Δ*catRBC*::P$_{tac}$-*catA* Δ*pcaHG*::*P$_{tac}$-aroY-ecdB-asbF* Δ*pykA*::*aroG^{D146N}-aroY-ecdB-asbF* Δ*pykF* Δ*ppc* Δ*pgi-1* Δ*pgi-2*	Glucose	Shake flask, batch	-	≈9.8	33.3	[31]
	P. putida KT2440	CJ522	CJ442 with Δ*gcd*	Glucose	Shake flask, batch	-	≈11.3	38.9	
				Glucose	Bioreactor, batch	-	37.3	41.3	
				Glucose	Bioreactor, fed-batch	-	84.4	37.8	
	P. putida KT2440	GB062	CJ522 with Δ*hexR*	Glucose	Shake flask, batch	-	8.5	33.7	[49]
					Bioreactor, fed-batch	-	110	≈35.0	
	P. putida KT2440	GB271	CJ522 with Δ*hexR* Δ*gacS* Δ*gntZ*	Glucose	Bioreactor, fed-batch	-	155	35.6	
	P. chlororaphis HT66	HT66-MA6	Δ*pcaGH* Δ*pca$_{α/β}$* Δ*catB* Δ*phzD*::*P$_{phz}$→xanB2* Δ*phzAB*::*catA-pobA-aroY* Δ*phzE* Δ*lon* Δ*rpeA* Δ*remE* Δ*pykA P$_{J}$→xanB2-phzC P$_{a}$→catA-pobA-aroY*	Glycerol	Shake flask, batch	20 g L^{-1} tryptone	18.3	19.3[b)]	[224]
				Glycerol	Shake flask, fed-batch	20 g L^{-1} tryptone	23.8	n.d.	
	P. taiwanensis VLB120	GRC3Δ8Δ*pykA-tap*Δ*catB attTn7::P$_{14f}$-phdBCDE-4cl-pal*	genome-reduced chassis, Δ*pobA* Δ*hpd* Δ*quiC* Δ*quiC1* Δ*quiC2* Δ*phhAB* Δ*katG* Δ*PVLB_10 925* *trpE^{P290S} aroF-1^{P148L} pheA^{T310I}* Δ*pykA*, Δ*catB attTn7::P$_{14f}$-phdBCDE-4cl-pal*	Glucose	Bioreactor, fed-batch	-	7.2	n.d.	[42]
2-pyrone-4,6-dicarboxylate	*P. putida* KT2440	CJ598	Δ*pykA*::*P$_{tac}$-aroG^{D146N}-asbF* Δ*pykF* Δ*ppc* Δ*pgi-1* Δ*pgi-2* Δ*pcaHG*::*P$_{tac}$-ligABC* Δ*gcd*	Glucose	Bioreactor, fed-batch	-	70.1	39.8	[31]
isocincho-meronate	*P. putida* KT2440	CJ599	Δ*pykA*::*P$_{tac}$-aroG^{D146N}-asbF* Δ*pykF* Δ*ppc* Δ*pgi-1* Δ*pgi-2* Δ*pcaHG*::*P$_{tac}$-praA* Δ*gcd*	Glucose	Bioreactor, fed-batch	-	0.36	2.7	[31]
picolinate	*P. putida* KT2440	CJ596	Δ*pykA*::*P$_{tac}$-aroG^{D146N}-asbF* Δ*pykF* Δ*ppc* Δ*pgi-1* Δ*pgi-2* Δ*pcaHG*::*P$_{tac}$-praAH* Δ*gcd*	Glucose	Bioreactor, fed-batch	-	0.41	5.6	[31]

(*Continued*)

Table 1. Continued.

Product	Host	Strain	Genotype, description	Substrate	Cultivation mode	Additives	Titer [mM]	Yield % [Cmol Cmol^{-1}]	Ref.
phenazine-1-carboxylate	*P. aeruginosa* PA1201	PA-IV	reduced virulence strain, Δ*phzM* Δ*phzS* Δ*phzH* Δ*pabBC* Δ*trpE* Δ*pchA* Δ5'UTR-*phzA1-G1* *pheA*^{W323L} *ubiC*::Rv2949c, deletion of 21 secondary metabolite gene clusters, overexpression of *aroG*, *phzC1*,*phzA2-G2*, and *mexGHI-opmD* (efflux pump)	Glucose	Shake flask, batch	22 g L^{-1} tryptone	20.1	n.d.	[33]
				Glucose/ ethanol	Shake flask, batch	65.02 g L^{-1}	35.2	n.d.	
				Glucose	Bioreactor, fed-batch	soybean meal, 15.36 g L^{-1} corn steep liquor	44.1	n.d.	
						30.0 g L^{-1} soybean meal, 7.5 g L^{-1} corn steep liquor, 10 g L^{-1} tryptone			
	P. putida KT2440	14.phz2	expressing *phzA2B2C2D2E2F2G2* from *P. aeruginosa* PA14	Glucose	Shake flask, fed-batch	-	1.30	n.d.	[74]
	P. chlororaphis GP72	ANOFK1	Δ*rpeA* Δ*phzO* Δ*mgsA*; overexpression of *glpF* and *glpK*	Glycerol	Shake flask, batch	20 g L^{-1} tryptone	6.7	n.d.	[225]
phenazine-1-carboxamide	*P. chlororaphis* HT66	P3	obtained through chemical mutagenesis and screening	Glycerol	Shake flask, batch	20 g L^{-1} tryptone	9.0	n.d.	[17]
	P. chlororaphis HT66	HT66LSP	Δ*lon*, Δ*parS* Δ*psrA*	Glycerol	Shake flask, batch	20 g L^{-1} tryptone	18.4	41.1[b]	[226]
	P. chlororaphis HT66	P3Δ*lon*	derived from *P. chlororaphis* HT66 P3 with Δ*lon*	Glycerol	Shake flask, batch	20 g L^{-1} tryptone, 25.03 g L^{-1} soy peptone	41.1	n.d.	[75]
2-hydroxy-phenazine	*P. chlororaphis* GP72	GP72ND-3 pbBb5k-*aroE-aroD-aroB-phzC-tktA-ppsA*	Δ*pykF* Δ*rpeA* Δ*rsmE* Δ*lon*; overexpression of *aroE*, *aroD*, *aroB*, *phzC*, *tktA*, and *ppsA*	Glycerol	Shake flask, batch	20 g L^{-1} tryptone	3.30	n.d.	[34]
DHHA	*P. chlororaphis* GP72	DA4	Δ*phzF* Δ*rpeA* Δ*pykF*; overexpression of *tktA* and *ppsA*	Glycerol	Shake flask, batch	20 g L^{-1} tryptone	32.1	n.d.	[50]
				Glucose/glycerol	Shake flask, batch	20 g L^{-1} tryptone	48.2	n.d.	
	P. chlororaphis GP72	DA6	DA4 with Δ*rsmE* and Δ*lon*	Glycerol	Shake flask, batch	20 g L^{-1} tryptone	49.3	n.d.	[227]
				Glycerol	Bioreactor, fed-batch	20 g L^{-1} tryptone	64.8	30.3[b]	
cinnabarinate	*P. chlororaphis* GP72	CA3	Δ*phzF* Δ*rpeA* Δ*pykF*; expression of DHHA dehydrogenase $CalB_3$ and phenoxazinone synthase CotA	Glycerol	Shake flask, batch	20 g L^{-1} tryptone, Cu^{2+}, H_2O_2	0.27	n.d.	[228]
				Glycerol	Shake flask, fed-batch		0.45	n.d.	
						20 g L^{-1} tryptone, Cu^{2+}, H_2O_2			
1-hydroxy-phenazine *N'* oxide	*P. chlororaphis* HT66	P3-SN	derived from *P. chlororaphis* HT66 P3 with Δ*phzH*::P_{phzS}-*phzS* Δ*pykA*::P_{phz}-*naphzNO1*	Glycerol	Shake flask, fed-batch	20 g L^{-1} tryptone	0.67	n.d.	[229]
2-acetamido-phenol	*P. chlororaphis* P3	P3Δ*phzB*Δ*lon* Δ*pykA*Δ*rsmE*	Δ*phzB* Δ*lon* Δ*rsmE* Δ*pykA* native expression of *nat* (N-acetyltransferase)	Glycerol	Shake flask, batch	20 g L^{-1} tryptone	4.82	n.d.	[76]
phenazine-1,6-dicarboxylate	*P. chlororaphis* GP72	GP72Δ*phzG*	Δ*phzG*	Glycerol	Shake flask, fed-batch	tryptone	0.25	n.d.	[230]
pyocyanin	*P. putida* KT2440	14.phz2+	expressing *phzA2B2C2D2E2F2G2* from *P. aeruginos*a PA14 and *phzMS* from *P. aeruginosa* PAO1	lucose	Shake flask, fed-batch	-	0.16	n.d.	[74]

Abbreviation: DHHA, trans-2,3-dihydro-3-hydroxyanthranilate; n.d., not determined. [a] Yield considering the additional non-glycerol carbon; [b] Medium with considerably high amounts of complex substances that were not considered for yield calculations.

efficient 4-hydroxybenzoate production (1.06 mM with 12.4% Cmol Cmol^{-1}) in *P. putida* S12pal_xylB7 after an evolutionary selection.[48] Interestingly, the same strain also showed enhanced 4-hydroxybenzoate production on glucose and glycerol compared to its progenitor indicating an overall increased flux through the PPP likely as a result of the adaptive laboratory evolution. These results emphasize the high potential of the non-oxidative xylose pathway and its higher suitability for aromatics production compared to the Weimberg and Dahms pathway. However, the latter pathways can be beneficial for split metabolism approaches, in which glucose is mainly used for product formation and xylose for biomass and energy generation. A corresponding approach has been demonstrated for *E. coli* using the Dahms pathway.[56]

2.1.4. Product Diversification

Once efficient *Pseudomonas* production strains of certain aromatics are obtained, they can usually be adapted to enable production of other valuable chemicals that are derived from common precursors. For instance, phenol-producing *P. putida* S12TPL3 has been adapted for the production of 4-hydroxybenzoate[23] and 4-vinylphenol[57] by replacing the product-specific biosynthetic module. Tyrosine-overproducing platform strains can also be adapted to obtain phenylalanine-derived products by the deletion of phenylalanine monooxygenase PhhAB and other reactions involved in phenylalanine degradation, because a substantial proportion of the flux to tyrosine is via phenylalanine.[14,25] This was used to convert the phenol-producing *P. taiwanensis* VLB120 into a *trans*-cinnamate producer.[25] In a subsequent study, the resulting strain was adapted to enable benzoate, catechol, and *cis,cis*-muconate synthesis from *trans*-cinnamate.[42] Molina-Santiago et al.[13] used phenylalanine-overproducing strain DOT-T1E CM12-5 to diversify the product spectrum by implementing modules for 2-phenylethanol and *trans*-cinnamate synthesis.

Despite the extensive progress that has been made, the de novo biosynthesis of bulk aromatics needs to be further improved regarding titer, rate, and yield to be able to compete with the current inexpensive petrochemical production. Especially the rate is one of the most limiting factors hindering a profitable bio-based production as has been paradigmally calculated for 4-hydroxybenzoate by Krömer et al.[58] The biocatalytic synthesis of structurally more complex aromatic fine chemicals might economically be more feasible due to the high regio- and stereoselectivity of enzymes.

2.2. Aromatic Secondary Metabolites

Besides rather simple aromatic commodity and bulk chemicals, the synthesis of high-value aromatic fine chemicals is an expanding field.[59–61] Pseudomonads are used as natural and heterologous secondary metabolite producers[62–64] and are treasure troves of enzymes involved in their biosynthesis.[65,66] Multiple gene clusters, coding for non-ribosomal peptide synthase (NRPS) or polyketide synthase (PKS) pathways reveal versatility for mining of new promising molecules[67] like dialkylresorcinols, carotinoids, acyl-polyenes,[68] and many more. Aromatic fine chemicals can derive from the shikimate pathway exclusively by condensations of respective aromatic precursors (e.g., phenazines), degradation or conversion of intermediates (e.g., pyrrolnitrin) or by incorporation with other precursors by NRPS (e.g., pyoverdines/siderophores, pyoluteorin). Alternatively, they can be formed by PKS, which catalyze a ring closure after condensation of acyl-extender units by Claisen- or aldol condensation (e.g., phloroglucinol, chalcones, stilbenes).[69] Thus, the formation of aromatics can be completely independent from the shikimate pathway, although the enzymes responsible are considered to be slower and more cumbersome.

2.2.1. Non-Polyketide Aromatic Secondary Metabolites

Pseudomonas spp. produce about 100 different phenazines from chorismate as a central metabolic intermediate.[70] Many of these have antibiotic properties,[17] but they can also function as redox mediators that enable interaction with electrodes for a reduced oxygen demand.[71,72] Phenazines production is regulated in a complex, quorum sensing-dependent manner.[70,73] Heterologous biosynthesis in *P. putida* KT2440 is highly dependent on the origin of the respective synthesis operon.[74] The highest titer with a rationally engineered natural producer for phenazine-1-carboxylic acid was achieved with *P. aeruginosa* PA1201 by elevating DAHP synthases expression, promoter exchange of two phenazine clusters and the transporter MexGHI, and by blocking 21 competing secondary metabolite clusters and limiting essential chorismate-consuming reactions. The resulting rationally engineered strain produced up to 9.9 g L^{-1} phenazine-1-carboxylate in a fed-batch fermentation.[33] In contrast, strain *P. chlororaphis* P3 obtained by mutagenesis and screening of *P. chlororaphis* HT66 reached a titer of 1.7 g L^{-1} phenazine-1-carboxamide.[17] Further optimization of the culture conditions enabled production of 9.2 g L^{-1} phenazine-1-carboxamide with *P. chlororaphis* P3 Δ*lon* in shake flasks.[75] Disrupting the phenazine pathway enabled the synthesis of 1.2 g L^{-1} 2-acetamidophenol in *P. chlororaphis* P3 Δ*phzB* due to a native arylamine *N*-acetyltransferase.[76] The hydroquinone glycoside arbutin is frequently produced by cell-free enzymatic conversion or in biotransformation processes.[77] The ability to perform glycosylation is a major advantage of eukaryotic hosts compared to prokaryotic hosts. Nevertheless, functional glycosylation for the synthesis of plant-derived metabolites by *P. chlororaphis* P3 was accomplished for arbutin. The respective genes of the pathway, starting from supplemented 4-hydroxybenzoate, were expressed including a glycosidase from the native promoter P_{phz}.[39]

Violacein is a vesicle-secreted antibiotic from various Gram-negative bacteria like *Chromobacterium violaceum*. It is a violet bisindole derived from tryptophan[78] and has been synthesized by *P. putida* strains by inserting the 7.4 kb operon from *C. violaceum* into random genomic loci by the yTREX system reaching up to 105 mg L^{-1}.[79] Pyrrolnitrin, another compound derived from tryptophan but natively occurring in *Pseudomonas* species, is an agricultural fungicide.[62,80,81] Production strains were traditionally generated by screening for analogue-resistant mutants.[82] Pyrrolnitrin is produced by various species with cooccurring ability of phloroglucinol biosynthesis[83] at various concentrations depending on the applied low cost fermentation substrate.[84]

Pseudomonads contain innate NRPS for the formation of siderophores, like pyoverdines and azotobactin.[85] Pyoverdines are made in the cytoplasm in response to iron-limiting conditions.[86] The formation of functional NRPS require the activation of an acyl carrier protein and peptidyl carrier protein domain by phosphopantetheinyl transferase (PPTase) which in case of *P. putida* KT2440 has a broad substrate spectrum. It thus allows activation without additional heterologous PPTase expression in contrast to other prokaryotes.[87,88] This allows functional expression of large NRPS of foreign origin,[89,90] enabling the production of, among others, 150 mg L^{-1} prodigiosin,[79] about 3 mg L^{-1} docosahexaenoic acid,[90] and 0.6 mg L^{-1} myxothiazol A.[89]

2.2.2. Aromatic Polyketides

There are also shikimate-independent sources of aromatic secondary metabolites like resorcinols and polyketides. Pyoluteorin is a native chlorinated antibiotic with comparable ring-formation like 2,5-dialkylresorcinol deriving from a NRPS/PKS hybrid pathway.[91,92] Significant improvement concerning pyoluteorin production was achieved by deleting transcriptional and translational repressors, Lon protease and regulatory sequences, as well as overexpression of the respective transport operon.[93]

Phloroglucinol, and its derivates monoacetylphloroglucinol and 2,4-diacetylphloroglucinol (DAPG) are naturally occurring polyketides from diverse *Pseudomonas* spp.[94,95] Their application as precursor of rocket fuels[96] and as an antibiotic raised early attention.[97] PhlD is a bacterial type III PKS, catalyzing the condensation of three malonyl-CoA (MaCoA) to phloroglucinol.[98] PhlACB is an acetyltransferase, able to form C–C bonds on aromatics[99] and PhlE is an exporter.[100] Nakata et al.[101] produced about 1.2 g L^{-1} DAPG with the natural producer *P. fluorescens* S272 after stress induction with a heat shock. Previously the use of ethanol as carbon source, high C/N ratios or applying high salt concentrations also increased titers.[102] PhlD from *P. fluorescens* Pf-5 was engineered for higher turnover numbers and decreased K_M,[103] as well as for higher thermostability to facilitate its use in different microorganisms.[104] Promising variants are $PhlD^{Y256R,A289R}$, $PhlD^{23D9}$, and $PhlD^{M21T,L54V,A82T,A181S}$ with improved properties, as well as PaP79 from Meyer et al.[96]

Flaviolin (2,5,7-trihydroxy-1,4-naphthoquinone) is a red compound derived from 1,3,6,8-tetrahydroxynaphthalene, a polyketide made from five MaCoA by a type III PKS of bacterial origin (RppA from *Streptomyces* spp., SoceCHS1 from *Sorangium* sp.).[105] It has been used to determine the MaCoA availability in a heterologous host to assess the potential for polyketide synthesis.[21,106] In early attempts to produce flaviolin in pseudomonads, ≈6 mg L^{-1} were achieved in *P. putida* KT2440.[107] In recent attempts, testing RppA variants and different concentrations of supplemented glucose to complex medium, 65 mg L^{-1} were produced with a truncated enzyme variant.[21] Yang et al.[106] produced 44.7 mg L^{-1} while addition of up to 100 µM cerulenin roughly doubled the titer in a dose-dependent manner.

Plant-derived polyketides can also partly be derived from an aromatic CoA-ester like cinnamoyl-CoA, benzoyl-CoA, or 4-coumaroyl-CoA as starter unit in combination with acyl-CoA extenders, which form a second phenyl group.[108–110] Synthesis of the plant metabolite bisdemethoxycurcumin (≈2 mg L^{-1}) was achieved by combining an incomplete natural phenylpropanoid degradation pathway with a heterologously expressed curcuminoid synthase.[21] Examples of chalcone or stilbene synthesis with *Pseudomonas* spp. as heterologous host are lacking thus far. However, export of polyphenols like naringenin and other compounds by RND-type efflux pump TtgABC from *P. putida* DOT-T1E, has been identified[111] indicating an interesting potential for this class of compounds. Moreover, MaCoA precursor supply was increased by deletion of *fabF* in *P. denitrificans*.[112] The previously mentioned *Pseudomonas* strains that efficiently synthesize precursors like *trans*-cinnamate[25] and benzoate,[42] would make ideal chassis for such polyketides.

3. Biotransformation

3.1. Aromatics Production with *Pseudomonas* in Solvent Two-Phase Fermentations: Featured and Empowered by Solvent-Tolerance

3.1.1. Solvent Tolerance and Toxicity

One unique feature of certain *Pseudomonas* spp. is their solvent tolerance. The mechanisms of solvent tolerance have been extensively reviewed,[113,114] as has the potential of solvent tolerance in biotransformation.[113,115–118] The main determining factors of solvent tolerance is extrusion of the toxic solvent by diverse energy-dependent efflux pumps.[119,120] The extrusion mediated by resistance-nodulation-division (RND) family transporters is driven by the proton motive force. Energy requirements of this and other tolerance mechanisms are provided by an increased catabolic capacity including an increased substrate uptake rate and elevated TCA cycle flux with a simultaneously reduced biomass formation according to the "driven by demand" concept in response to solvent stress.[43,121,122] Other stress response genes are induced for protein refolding and scavenging of reactive oxygen species.[123] Additionally, *cis-trans*-isomerization of lipids in the cell membrane by Cti increases the membrane rigidity to counteract its destabilization caused by the accumulation of solvent in the membrane. This short-term mechanism is supported by an increased embedment of de novo-synthesized saturated fatty acids into the phospholipid bilayer as a longer-term response.[124]

The application of biphasic liquid-liquid fermentations with a hydrophobic second phase serving as extractant integrates downstream processing into the production process.[117] The use of solvent-tolerant strains offers a wider degree of freedom regarding the application of suitable solvents with desired product extraction qualities and phase separation characteristics to simplify product recovery and purification. Such systems provide in situ extraction of toxic products to reach high product concentrations, and they can also contain a reservoir of toxic substrate, negating the need for complex fed-batch strategies. The latter strategy is applied in several studies with *Pseudomonas* spp. in production processes of aromatic compounds (**Table 2**). Besides classical organic solvents, ionic liquids can also be used for in situ recovery of aromatics,[125] although their high price is often a major hurdle. The specific selection of solvents for their intended application[126] or the use of solvents in pertraction processes with membranes are used.[127,128] The determination of a

Table 2. Studies using pseudomonads in biphasic cultivations for the production of aromatics and derived compounds.

Aim/Product	2nd organic phase	Host	Note	Ref.
3-methylcatechol	Octanol	*P. putida* S12	Implementation of *todC1C2BAD* from *P. putida* F1; two-fold improvement by biphasic fermentation	[170]
	Octanol	*P. putida* MC2	50% (v/v) octanol elevated titer from 10 mM to 25 mM	[172]
	Octanol	*P. putida* MC2	Specific productivity of 235 μmol min^{-1} g$^{-1}_{CDW}$ at pH 6	[231]
	Octanol, nonanol, decanol	*P. putida* DOT-T1E	*m*-xylene to 3-methylcatechol by incomplete degradation pathway; 70 mM 3-methylcatechol when decanol was used	[173]
	bis(2-ethylhexyl) sebecate	*P. putida* MC2	Determination of logP_{crit} for host is 3.1; partitioning coefficient of 3-methylcatechol for various solvents; volumetric productivity is 440 mg L^{-1} h^{-1}	[138]
	Oleyl alcohol	*P. putida* T-57	Substrates are glucose and butanol; 107 mM product in organic phase; doubled overall titer compared to single phase cultivation	[174]
6-β-hydroxycholest-4-en-3-one cholest-4-ene-3,6-dione	1,4-xylene and diphenylmethane (3:7)	*P.* sp. ST-200	Transformation of water-insoluble cholesterol in aromatic solvent	[232]
o-cresol	Octanol and toluene (7:3)	*P. putida* T-57	Isolated strain tolerant till log$P_{O/W}$ 2.5; 1.7 g L^{-1} product in aquatic-phase	[175]
4-hydroxybenzoate	Toluene	*P. putida* DOT-T1E	*pobA* and *todC* deletion strain; heterologous pathway with *tmo* and *pcu* from *P. mendocina*	[176]
4-vinylphenol	*n*-decanol	*P. putida* S12	Titer increase due to product toxicity from conversion	[57]
Phenol	*n*-octanol	*P. putida* S12	Titer increase due to product toxicity from conversion	[9]
(*S*)-styrene oxide	AL240 (iso-, cyclo-, and linear alkanes with a chain length of at least 13 carbon)	*P. putida* KT2440	Heterologous use of XylMA and StyAB; continuous biphasic cultivation stable over 100 generations (350 h)	[164]
	bis(2-ethylhexyl)phthalate (BEHP)	*P. putida* SN1	Copy number of *styAB* is not rate-limiting	[165]
	BEHP	*P. taiwanensis* VLB120	Styrene concentration is limiting its conversion	[166]
	Octanol; BEHP	*P. putida* DOT-T1E	Maintenance for octanol limits NADPH availability for redox catalysis	[43]
	Toluene; styrene	*P. taiwanensis* VLB120	Constitutive TtgGHI expression elevates stereospecific styrene epoxidation	[167]
	BEHP	*P. taiwanensis* VLB120	Reduction of 89% and 56% of BEHP is achieved while balancing volumetric productivity and environmental impact	[168]
	BEHP	*P. taiwanensis* VLB120	Glucose excess elevates specific activity	[169]
Toluene *cis*-glycol fluorocatechol	Tetradecane	*P. putida* UV4 *P. putida* ML2	Use of membrane oxygenator	[233]

suitable biocompatible solvent is a major task for the application of two-phase bioconversions and -transformations.[129,130] Here, we focus on hydrophobic, highly toxic products and substrates in bioconversion and biotransformation using whole cells of *Pseudomonas* in combination with organic solvents.

The choice of organic solvent and microbial host is a decisive for the overall production process.[131] The range of biocompatible solvents strongly depends on the selected host organism. Many organic solvents are toxic to cells due to their solubility in cell membranes. There, they change membrane fluidity,[132] lead to permeabilization or swelling, and affect membrane proteins.[114] The log$P_{O/W}$, the logarithmic partitioning coefficient of water and octanol, is used as a reference for the hydrophobicity of a solvent.[133] It is also directly correlated to the partitioning of a solvent between a buffer solution and biological membranes (log$P_{M/B}$) by an equation from Sikkema et al.[134]

$$\log P_{M/B} = 0.97 \times \log P_{O/W} - 0.64 \quad (1)$$

Because of this, the physical parameter log$P_{O/W}$ allows estimation of the level of toxicity of a solvent.[114] Values of log$P_{O/W}$ between 1 and 4 are generally considered as toxic for microorganisms[135] since they exceed a critical concentration in membranes in the range of 400 mM.[136] The determination of the maximum membrane concentration (MMC), which considers log$P_{M/B}$ and solubility of compound in the aqueous phase (S_{aq}), is an accurate predictor of solvent cytotoxicity.[114,115,136]

$$MMC = S_{aq} \times 10^{\log P_{M/B}} \quad (2)$$

Besides MMC the determination of a respective $\log P_{crit}$ for various strains and solvents also offers a rational base for the selection of a suitable microbial host-solvent system.[137–139] Despite the use of monomeric solvents it is likely that this is also valid for low molecular weight polymers.[140]

3.1.2. Process Design of Biphasic Fermentations: Issues to be Considered

Although biphasic fermentations can provide several advantages, the use of solvents or the production of such also comes with specific process-oriented considerations. Particular attention regarding safety requirements in aerated fermentation processes may be needed due to flammability and risk of explosion. Multiple tools simplify the selection of adequate and safe extraction solvents although they are intended mainly for purification processes rather than in situ product recovery in highly aerated biological processes.[141] The oxygen mass transfer coefficient (k_La) is positively influenced by a hydrophobic phase, allowing facilitated oxygen supply for aerobic processes.[142] Solvents with low vapor pressure, auto-ignition temperature, and boiling temperature are recommended and processes with elevated pressure, low temperatures, and small amounts of solvent and oxygen concentration outside of the explosive range are desirable. Octane for instance, should be applicable when the fermentation process is run at 30 °C and at least 4.9 bar[143] which is possible in high-pressure, explosion-proof bioreactors.[144] The reduction of flammability and explosion is in contradiction to elevated risks from a pressurized process which has different safety concerns and effects on the biological system,[145] requiring special equipment depending on the applied solvent and process parameters. Other advantages of pressurized fermentations are increased biomass formation due to elevated oxygen solubility.[146,147] However, considerations about health concerns, corrosion of the equipment due to the applied chemicals and waste disposal should be made. Subsequent downstream processes rely on centrifugal separation, de-emulsifiers, temperature shifts, or catastrophic phase inversion.[148] Lastly, care should also be taken that the applied extractant is not inadvertently degraded by *Pseudomonas*,[9] as solvent losses have a major impact on process economy and environmental impact.

3.2. Biphasic Fermentation Processes with *Pseudomonas*

Biphasic reaction systems with organic solvents are often described as an option to facilitate the degradation of toxic water-insoluble xenobiotics[149–152] like α-pinene by *Pseudomonas fluorescens* NCIMB 11 671,[153] benzene, toluene, 1,4-xylene degradation by *Pseudomonas* sp. ATCC 55 595[154] or phenol by *P. putida* ATCC 11 172.[155] Pseudomonads have been applied in two-phase fermentations production processes for decades, ever since Schwartz and McCoy[156] performed transformations with *P. oleovorans* in presence of cyclohexane. The use of linear alkanes as second phase and substrate for production of the respective oxidation products was applied regularly[157,158] and was assessed for its economic potential 30 years ago.[159]

In the context of aromatics, the oxidation of styrene to (*S*)-styrene oxide with *Pseudomonas* has also been an ongoing research field ever since the 1990's. During this time, the discovery of *P. putida* S12 growing on styrene in a styrene-water system was made,[160] and usage of styrene degradation genes from, for example, *P. taiwanensis* VLB120 in traditional hosts[161–163] has shifted toward the direct application of the solvent-tolerant pseudomonads themselves (Table 2). *P. putida* KT2440 carrying a xylene monooxygenase was successfully incubated over 350 h, corresponding to 100 generations, in presence of a mixture of alkanes in a second phase.[164] Bae et al.[165] used the styrene degrader *P. putida* SN1 with a disrupted degradation pathway for the oxidation of styrene to (*S*)-styrene oxide for enantiopure biotransformation without the native expression of the styrene monooxygenase being rate-limiting. While a constitutive solvent-tolerant *P. taiwanensis* VLB120 as a host outperformed a heterologous *E. coli* host overexpressing the styrene monooxygenase genes, oxidation is limited by the applicable styrene concentration in the organic phase of bis(2-ethylhexyl)phthalate.[166] The influence of the solvent on maintenance and NADPH availability for redox catalysis with whole cells was elucidated in *P. putida* DOT-T1E.[43] The construction of a constitutive solvent-tolerant strain of *P. taiwanensis* VLB120[167] and subsequent reduction of required organic solvent[168] yielded an oxidation process with moderate specific activity and volumetric productivity with simultaneously reducing the environmental impact. Analysis of the respective substrate kinetics revealed that excess of glucose results in increased specific activity of the oxidation up to 180 U $g_{CDW}{}^{-1}$.[169]

Another frequently applied oxidation process is the transformation of toluene to 3-methylcatechol with operon *todC1C2BAD* encoding toluene dioxygenase and *cis*-toluene dihydrodiol dehydrogenase from *P. putida* F1 or DOT-T1E. Wery et al.[170] introduced the operon into *P. putida* S12 and revealed a reverse correlation between the concentration of added toluene and the 3-methylcatechol yield. Here, the second organic phase had an additional beneficial effect of preventing polymerization of 3-methylcatechol to a brownish precipitate, thereby avoiding a loss of product. Strain improvement of *P. putida* F1 to mutant F107 and subsequent chromosomal multicopy insertion of *todC1C2BAD* enabled a 3-methylcatechol titer of 14 mM with a rate of 105 μmol min^{-1} $g_{CDW}{}^{-1}$ in strain MC2 without requiring supplementation of antibiotics.[171] Application of a second phase of octanol elevated the titer further to 25 mM, with the ratio of the liquids playing an important role.[172] Keeping the organic phase separate of the fermentation broth appeared beneficial under the selected conditions.[127] An important step toward the rational design of fermentations with *Pseudomonas* in biphasic partitioning bioreactors was demonstrated by Prpich and Daugulis,[138] who initially determined $\log P_{crit}$ of the respective host MC2 and the partitioning coefficient K of 3-methylcatechol with a respective library of solvents. Based on these evaluations a selection of an organic solvent increased the volumetric productivity of 3-methylcatechol by about fourfold to 440 mg L^{-1} h^{-1} and reduced substrate loss of toluene by about fourfold as well. The overall maximal product titer of 5.5 g L^{-1} was limited by applicable volume and capacity of the solvent (the partitioning coefficient), which is better for aliphatic alcohols.[138] Usage of 1,3-xylene by an incomplete degradation pathway in *P. putida* DOT-T1E offers on opportunity to yield different alkylcatechols. Due to the fact that 3 mM 3-methylcatechol fully inhibited growth, cultivation with 50% (v/v) octanol or decanol was performed and allowed

the biosynthesis of 17 mM (2.6 g L^{-1}) or 70 mM (10.7 g L^{-1}), respectively.[173] Octanol had a fivefold higher negative impact on cell viability of the applied strain than decanol, explaining the higher overall titer despite a putative smaller partitioning coefficient.[173] The *tod* operon of *P. putida* T-57 is controlled by catabolite repression in the presence of glucose. Cosubstrates like butanol as an alternative carbon source allowed toluene transformation under non-repressive conditions reaching titers of up to 107 mM 3-methylcatechol in the oleyl alcohol phase.[174] Similar strategies to circumvent product toxicities and reach higher titers were applied to multiple other production processes (Table 2) of aromatics like *o*-cresol,[175] 4-hydroxybenzoate,[176] 1-naphtol,[177] and aliphatic products.

Most examples of biphasic fermentations are whole-cell biotransformations, consisting of one or two enzymatic steps, often relying on cellular metabolism to regenerate redox cofactors. This is mainly because in many cases the substrate is (also) hydrophobic, and the second phase acts as a substrate reservoir which keeps the aqueous concentrations below toxic levels. The application of de novo biosynthesis of aromatics in combination with an organic phase is relatively rare. One example was the abovementioned bio-based production of phenol with *P. putida* S12TPL3. Octanol was used as a second phase to extract phenol from the fermentation broth, almost doubling the phenol titer to 9.20 mM compared to 5.01 mM in a monophasic fermentation.[9] The strain was also used with solvent-impregnated resins,[125] aqueous poloxamer solutions,[178] and membrane separation[128,179] for phenol recovery. Application of decanol as second phase in a 4-vinylphenol producing derivative of this strain reduced the effect of product toxicity, elevating the titer to 21 mM and doubling volumetric productivity.[57] It should be noted that these totals concentrations are calculated for the combined liquid volumes of water and extractant, and that the concentrations in the organic phase reached much higher values of 58 mM phenol[9] and 147 mM 4-vinylphenol.[57]

An economic evaluation of a continuous in situ pertraction process for phenol production using a *Pseudomonas* resulted in costs of 18 € kg^{-1}, which is at least 20-fold higher than those of the chemical process, with the pertraction unit being the major cost-driving factor which is highly dependent on the mass transfer and thus on the selected extractant.[179] An integrated pertraction also requires large distillation columns and a high energy input for solvent regeneration due to constantly low product concentrations. A non-integrated in-stream product recovery approach from a fed-batch cultivation resulted in threefold increased costs (≈57 € kg^{-1}) mainly due to higher investment costs of the larger reactor due to lower space-time yield, highlighting the advantage of in situ product removal.[179] Beside the opportunity for different sales strategies to obtain higher prices for products of biological origin, it should be noted that products of higher value or the upcycling of waste streams could potentially enable a profitable bio-based process at lower productivities.

3.3. Application of Biotransformation for Production of Aromatics-Derived Compounds

The product of a biotransformation is often an intermediate of natural degradation, which is not further metabolized like 5-(hydroxymethyl)furfural to 2,5-furandicarboxylic acid[180] or tyrosol to hydroxytyrosol.[181] Additionally, pseudomonads often serve as source of oxygenases, dehydrogenases, or other enzymes for the synthesis of valuable aromatics.[162,182] D-4-Hydroxyphenylglycine, an aromatic amino acid used for synthesis of semisynthetic antibiotics, is industrially produced in a dynamic kinetic resolution manner using a hydantoin racemase in combination with a D-hydantoinase and a D-*N*-carbamoylase[183] from diverse *Pseudomonas* spp.[184,185] This "hydantoinase process" is applicable to yield various D-amino acids replacing the conventional chemical synthesis and commercialized by several companies.[186]

Exploiting natural resistance for transformation was also used for other cytotoxic compounds like terpenes and monoterpenoids recently,[187] outperforming other hosts like *Saccharomyces cerevisiae* which required the addition of solvents that are more biocompatible.[188] Application of adsorbent and membrane oxygenation in separate loops to increase fluorocatechol production by *P. putida* was established by Lynch et al.[189]

Incomplete benzoate catabolism by BenABC, lacking BenD, yields the chiral intermediate compound *cis*-1,2-dihydroxycyclohexa-3,5-diene-1-carboxylate from benzoate in *P. putida* KT2442. A titer of 2.3 g L^{-1} and a yield of 73% (mol mol^{-1}) were achieved in batch cultures when BenABC was overexpressed. Application of a fed-batch increased the titer to 17 g L^{-1} with a rate of 0.356 g L^{-1} h^{-1}.[190] Other *cis*-diols are produced from benzene, toluene, and chlorobenzene with toluene dioxygenase by different *P. putida* KT2442 strains. As the yield of the these dioxygenase reactions are highly dependent on oxygen availability, besides traditional methods like increased airflow, agitation, and providing pure oxygen, simultaneous expression of *Vitreoscilla* hemoglobin protein elevated the microbial oxygen utilization rate under low dissolved oxygen conditions, increasing titers significantly.[191]

The use of essential oils like eugenol as substrate[192,193] and later lignocellulosic waste streams from pulp industry like ferulic acid[194] is exploited with *Pseudomonas* to obtain "natural" fragrances and flavors like vanillin and its derivatives for the food and beverages industry. Using and modifying the ferulate degradation cluster (*fcs-ech-vdh*) for vanillin synthesis with *P. fluorescence* BF13 yielded 8.41 mM vanillin.[195] The diverse opportunities to obtain vanillin from various substrates and the use of metabolically engineered hosts is summarized by Kaur and Chakraborty.[196] Further, to establish pseudomonads as hosts, proteome analysis of *P. putida* KT2440's response to vanillin showed that *vdh* is not necessarily required while solvent tolerance mechanisms are induced.[197] Subsequent engineering approaches of a plasmid-free producer showed that molybdate-depending oxidoreductases may accept vanillin as a substrate complementing the *vdh* inactivation. The final strain reached high ferulate transformation of up to 86% (mol mol^{-1}).[198] As food applications often demand non-GMO strains, strains obtained by, For example, UV mutagenesis provide a cumbersome but legally accepted alternative to produce vanillin and coniferyl aldehyde.[199] Purification of vanillin can be carried out in packed bed reactors for in situ product removal independent of the used host.[200] Food waste streams including ferulate were successfully converted to vanillic acid by engineered *P. putida* KT2440.[201]

Like ferulate, also many other lignin-derivable aromatics are attractive carbon sources since lignin is the second most

Table 3. Bioconversions for the production of value-added compounds derived from aromatic plastic monomers.

Product	Host	Strain	Genotype, description	Substrate	Cultivation mode	Carbon source	Titer	Yield	Ref.
PHA	*P. putida* F1	Wild type	Wild type	Benzene	Shake flask, batch	Benzene	0.05 g L^{-1}	n.d.	[218]
				Toluene	Shake flask, batch	Toluene	0.16 g L^{-1}	n.d.	
				Ethylbenzene	Shake flask, batch	Ethylbenzene	0.10 g L^{-1}	n.d.	
	P. putida mt-2	Wild type	Wild type	Toluene	Shake flask, batch	Toluene	0.08 g L^{-1}	n.d.	
				1,4-xylene	Shake flask, batch	1,4-xylene	0.14 g L^{-1}	n.d.	
	P. putida CA-3	Wild type	Wild type	Styrene	Shake flask, batch	Styrene	0.26 g L^{-1}	n.d.	
	Mixed culture of *P. putida* F1, *P. putida* mt-2, and *P. puida* CA-3	Wild types	Wild types	Benzene, toluene, ethylbenzene, 1,4-xylene, styrene	Shake flask, batch	Benzene, toluene, ethylbenzene, 1,4-xylene, styrene	0.25 g L^{-1}	n.d.	
				Benzene, toluene, ethylbenzene, 1,4-xylene, styrene	Shake flask, fed-batch	Benzene, toluene, ethylbenzene, 1,4-xylene, styrene	6.02 g L^{-1}	n.d.	
	P. putida CA-3	Wild type	Wild type	Styrene oil	Shake flask, batch	Styrene oil	0.14 g L^{-1}	62.5 mg g^{-1}	[219]
				Styrene	Shake flask, batch	Styrene	0.18 g L^{-1}	n.d.	
				Styrene oil	Bioreactor, fed-batch	Styrene oil	0.32 g L^{-1}	n.d.	
	P. sp. GO16 *P.* sp. GO19 *P.* sp. GO23	Wild types	Wild types	Terephthalate	Shake flask, batch	Terephthalate	≈0.25 g L^{-1}	n.d.	[220]
	P. sp. GO16	GO16 KS3	Evolutionary selection for growth on EG	Ethylene glycol / terephthalate	Bioreactor, batch	Ethylene glycol/terephthalate	0.15 g L^{-1}	n.d.	[214]
HAA	*P.* sp. GO16	GO16 KS3 pSB01	GO16 KS3 expressing *rhlA*	Ethylene glycol / terephthalate	Shake flask, batch	Ethylene glycol/terephthalate	35 mg L^{-1}	n.d.	[214]

Abbreviations: HAA, (3-hydroxyalkanoyloxy)alkanoate; PHA, polyhydroxyalkanoates; n.d., not determined.

abundant renewable polymer on earth and the most abundant aromatic substrate. However, until now, lignin is underutilized due to its challenging depolymerization and its valorization is an intensively studied field of research.[202] The production of lignin-derivable compounds using different microbes including *Pseudomonas* as biocatalysts has been extensively reviewed.[202,203] Among a few other genera, pseudomonads are promising hosts due to their degradative capacity and tolerance toward lignocellulosic inhibitors.[204,205]

The targeted disruption of native degradation pathway and/or the implementation of specific heterologous reactions give access to a plethora of valuable compounds as extensively demonstrated by the study of Johnson et al.[31] One major model compound of industrial relevance whose production has been demonstrated in many studies from varying aromatic substrates is *cis,cis*-muconate.[206–208] This dicarboxylate is particularly interesting for the polymer industry as it can be chemically converted to adipate, a compound that is copolymerized with hexamethylene diamine to yield nylon 6,6.[209] Complete lignin valorization chains using *cis,cis*-muconate-producing *P. putida* KT2440 as biocatalysts yielding bio-nylon were described by Vardon et al.[209] and Kohlstedt et al.[208] Recently, a completely bio-based transformation of lignin-derivable monomers (4-coumarate, ferulate, 4-hydroxybenzoate) to adipate was demonstrated using *P. putida* KT2440 by introducing an artificial pathway that converts the naturally occurring degradation intermediate β-ketoadipate into adipate with titers and yields up to 17.4 mM and 18.4% mol mol^{-1}.[210] This process shows a high potential but is currently somewhat limited by by-product formation.[210] The popularity of *cis,cis*-muconate is related to its value but also because it can be derived from many different substrates. In *Pseudomonas*, *cis,cis*-muconate is a natural intermediate of benzoate, phenol, and catechol degradation. Many other aromatics are converted to protocatechuate and would naturally bypass this intermediate. However, through inactivation of the protocatechuate degradation pathway and simultaneous integration of protocatechuate decarboxylase, many other aromatic substrates present in lignin hydrolysate (e.g., 4-coumarate, ferulate, 4-hydroxybenzoate) can be funneled to catechol and subsequently to *cis,cis*-muconate. Thus, metabolic funneling of heterogonous mixtures of many different lignin-derivable compounds to a common product is attractive as this facilitates product isolation and purification that is also applicable to non-aromatic products including polyhydroxyalkanoate (PHA)[22,211,212] or organic acids including PYR and lactate.[213]

3.4. Anthropogenic Waste Streams as Carbon Source

Analogous to the valorization of natural lignin, anthropogenic plastic polymer waste streams are promising resources for the production of value-added chemicals due to their high abundance with 359 million tons produced in 2018 with only a minor fraction being recycled.[214] Many plastics, including polyethylene terephthalate (PET) and polystyrene, consist of aromatic monomers. Their enzymatic or thermochemical depolymerization make the monomers available for biotechnological

applications. Subsequent microbial valorization has a huge potential to integrate waste management into the production of value-added chemicals by establishing plastics waste as a carbon source for biotechnology.[213] This includes the upcycling of recalcitrant petrochemical polymers into biodegradable bio-based plastics such as PHA and polylactate.[215,216] Due to their metabolic versatility toward aromatic and non-aromatic plastic monomers *Pseudomonas* spp. are appealing hosts for plastic upcycling (**Table 3**).[217]

Benzene, toluene, ethylbenzene, xylene, and styrene (BTEXS) are versatile building blocks and constitute the major aromatic fraction of petroleum, making them common pollutants of the petrochemical industry. Moreover, BTEXS aromatics appear in pyrolyzed mixed plastic waste oils. Different *Pseudomonas putida* wild type strains were applied for the conversion of BTEXS to PHA.[218] In Ward et al.,[219] styrene was derived from polystyrene by efficient pyrolysis. The obtained styrene oil was used as sole carbon and energy source in a nitrogen-limited fermentation of *P. putida* CA-3 with a production of 0.32 g L^{-1} PHA. Likewise, terephthalate was obtained by PET pyrolysis and used as carbon source for the PHA production using different *Pseudomonas* strains naturally catabolizing terephthalate.[220] The pyrolytic treatment of plastic polymers is an energy-intensive process requiring high temperatures and pressures.[219,220] A biological depolymerization is challenging due to the high recalcitrance of plastics but would be advantageous. Especially the enzymatic hydrolysis of PET is currently an intensively investigated field of research[221,222] to make plastic monomers accessible. Recently, Tiso et al.[214] used PET that was enzymatically converted into its monomers, ethylene glycol and terephthalate, by a thermostable polyester hydrolase with a yield of 100%. Subsequently, *Pseudomonas* sp. GO16 was applied to produce either intracellular PHA or the extracellular building block (3-hydroxyalkanoyloxy)alkanoate. While the first has a potential application as bioplastic, the latter was copolymerized to a novel bio-based poly(amide urethane).[214] In this approach terephthalate and ethylene glycol were both fully metabolized to produce non-aromatic plastics. However, aromatic plastic monomers can also be transformed into other aromatics or derived compounds by the abovementioned funneling approach. Thus, strategies and pathways applied for the valorization of lignin-derived aromatics are also applicable for the valorization of plastic-derived aromatics.

4. Conclusions

Pseudomonads have long been lauded for their versatile metabolism. In this review, this versatility surrounding the conversion and/or production of aromatic compounds becomes readily apparent. Several decades of fundamental and application-oriented research on *Pseudomonas* as biotransformation host has provided a wealth of information on its metabolism, as well as on its unique tolerance to chemical stresses and the process-associated advantages this can bring. This research, coupled to intensive development of molecular tools, paves the way for increasingly intricate strain designs that enable more complex conversions including the high-yield de novo production of aromatics from renewables feedstocks, and the funneling of depolymerized lignin and plastic to value-added products. Looking forward, we expect an even further expansion of the range of metabolizable substrates of *Pseudomonas* to encompass complex hydrolysate mixtures, as well as an expanding product range including increasingly complex aromatics and related secondary metabolites. Extensive modifications of the organism itself will further facilitate the establishment of *Pseudomonas* as microbial cell factory by moving from *ad hoc* utilization of environmental isolates to the *à la carte* engineering and selection of chassis strains that are tailored to specific products and processes.

Acknowledgements

The authors acknowledge funding from the German Federal Ministry of Education and Research (BMBF) for the project NO-STRESS (FKZ 031B0852A) and from the German Federal Environmental Foundation (DBU) through the PhD Scholarship 20019/638-32.

Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Not applicable

Keywords

aromatics, biocatalysis, metabolic engineering, *Pseudomonas*, renewable resources

Received: June 30, 2020
Revised: September 8, 2020
Published online: October 20, 2020

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