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Transcriptome profiling of *Paraburkholderia aromaticivorans* AR20-38 during ferulic acid bioconversion

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Abstract

The importance and need of renewable-based, sustainable feedstocks increased in recent years. Lignin-derived monomers have high potential, energetic and economic value in the microbial bioconversion to valuable biomolecules. The bacterium *Paraburkholderia aromaticivorans* AR20-38 produces a remarkable yield of vanillic acid from ferulic acid at moderate and low temperatures and is therefore a good candidate for biotechnological applications. To understand this bioconversion process on a molecular level, a transcriptomic study during the bioconversion process was conducted to elucidate gene expression patterns. Differentially expressed genes, cellular transporters as well as transcriptional factors involved in the bioconversion process could be described. Additional enzymes known for xenobiotic degradation were differentially expressed and a potential membrane vesicle mechanism was detected. The bioconversion mechanism on a transcriptional level of *P. aromaticivorans* could be elucidated and results can be used for strain optimization. Additionally, the transcriptome study showed the high potential of the strain for other degradation applications.

Keypoints

- *P. aromaticivorans* AR20-38 differentially regulates three main enzymes for the bioconversion of ferulic acid to vanillic acid.
- ATP binding cassette transporters, major facilitator superfamily transporters and outer membrane vesicles are putative transport routes for catabolic enzymes.
- Regulated degradation enzymes indicate xenobiotic degradation potential

Keywords: Ferulic acid, Vanillic acid, Transcriptome, Bioconversion, *Paraburkholderia*

Introduction

Lignin is the second most abundant component of plants. It is a three-dimensional complex molecule consisting of various aromatic compounds. To enter the carbon cycle, lignin is degraded to lignin monomers by extracellular

enzymes of bacteria and fungi (Bugg et al. 2011). Subsequently, less complex aromatic compounds can be used as carbon source by other microbes. For example, lignin-derived bi- and monoaryls can be degraded by bacteria (Kamimura et al. 2017). These processes are crucial for ecosystem functioning of the natural environment and may be utilized in the valorization of renewable feedstocks for industrial applications. The substitution of fossil fuel-based to renewable-based, nonfood feedstocks has been suggested as sustainable solution for

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environmental problems (Anastas and Eghbali 2010). This leads to an increased interest in lignin valorization as well as biocatalytic bioconversion of e.g. ferulic acid (FA) as feedstock to produce valuable biomolecules (Cao et al. 2018; Civolani et al. 2000; Graf and Altenbuchner 2014; Mori et al. 2021). Moreover, microorganisms being good degraders of aromatic compounds could harbor applicable enzymes for pollutant or synthetic polymer degradation (Fuchs et al. 2011; Knott et al. 2020).

Within this group of promising microbial candidates to be utilized for such applications are members of the genus *Paraburkholderia*. *Paraburkholderia aromaticivorans* BN5^T is a hydrocarbon degrading bacterium, which was previously isolated from gasoline-contaminated soil (Lee and Jeon 2018). Berger et al. (2021) described the isolation of the strain *Paraburkholderia aromaticivorans* AR20-from Alpine forest soil. The microorganism was able to utilize a high amount of lignin sulfonic acid and phenol as sole carbon source (Berger et al. 2021). The cold adapted, Gram-negative bacterium belongs to the group of *Betaproteobacteria* and exhibits its degradation capability over a wide range of temperatures (0–30 °C). A rich genomic toolkit enables the strain to express various enzymes capable of degrading additional lignin monomers, including *p*-coumaric acid, 4-hydroxybenzoic acid, FA, vanillic acid (VA) and benzoic acid (Margesin et al. 2021; Poyntner et al. 2020). The high bioconversion capacity (88–89%) of FA to vanillin and further to VA at low (10 °C) and moderate (20 °C, 30 °C) temperatures is particularly interesting for biotechnological applications. A comparable high yield from pure FA was previously only reported from an engineered *Pseudomonas putida* strain KT2440 (Upadhyay et al. 2020).

The degradation of FA to vanillin has been intensively studied in different microbes due to the utilization of vanillin as a flavoring agent (Graf and Altenbuchner 2014). Additionally, there is a growing interest in the microbial production of medium chain-length polyhydroxyalkanoates using FA as a non-fatty acid feedstock. Therefore, the improvement was studied in *P. putida* using CRISPR/Cas9 (Zhou et al. 2020). To date, however, studies on cold-adapted strains are scarce even though these strains may be of particular interest for cost-efficient applications in a number of circumstances where maintaining high process temperatures is not a viable option.

To elucidate the gene expression patterns during the bioconversion of FA to VA in *P. aromaticivorans* we here investigated the transcriptomic response in the exponential growth phase with FA as sole carbon source. Therein, the differentially expressed genes responsible for the individual bioconversion stages of FA to VA in *P. aromaticivorans* were identified and putative

transcription factors and transporters were linked to the FA bioconversion.

Materials and methods

Experimental setup

The experimental setup based on two different culture conditions: the bacterial strain *P. aromaticivorans* cultured with glucose or FA as sole carbon source. The transcriptome during the bioconversion was sequenced and the differential expression during cultivation with FA in comparison to glucose was determined. The bioconversion was monitored with high pressure liquid chromatography (HPLC) measurements.

Culture conditions and RNA extraction

The experimental setup was based on the bacterial strain *P. aromaticivorans* cultured with three cultures with glucose and three cultures with FA as sole carbon source. Subsequently, from each culture RNA was extracted resulting in six RNA extracts which were further sequenced. The bioconversion to VA was monitored with HPLC. The strain *P. aromaticivorans* AR20-38 (deposited at China General Microbiological culture collection center under the number CGMCC 1.18749) was precultured in 20 mL pH-neutral mineral medium (MM: 3.5 g L⁻¹ Na₂HPO₄ × 2H₂O, 2 g L⁻¹ KH₂PO₄, 1 g L⁻¹ (NH₄)₂SO₄, 0.2 g L⁻¹ MgSO₄ × 7 H₂O, 0.05 g L⁻¹ Ca(NO₃)₂ × 4 H₂O, 10 mg L⁻¹ ammonium iron(III) citrate, a trace element and vitamin solution) (Margesin and Schinner 1997) containing glucose (2 g L⁻¹). After 3 days at 20 °C and 150 rpm the biomass was centrifuged (10 000 × g, 10 min), washed twice with sterile MM and resuspended in MM. 20 mL MM containing (i) 2 g L⁻¹ glucose (G1-3) or (ii) 10 mM trans-FA (Sigma-Aldrich 128708, FA1-3) were prepared. These were inoculated with the washed biomass at an initial (t₀) optical density at 600 nm (OD₆₀₀) of 0.05 in triplicates. The cultures were incubated at 20 °C and 150 rpm.

To determine the exponential phase, growth curves at the above described conditions were studied in pre-tests. Cells were harvested on ice in their exponential phase: G1-3 after 18 h and FA1-3 after 96 h. Approximately 5 × 10⁸ cells were centrifuged (4 °C, 10 000 × g) and washed twice with sterile MM. The supernatant was discarded, and 1 mL Nucleo Protect (Macherey–Nagel, Düren, Germany) was added before storing in liquid nitrogen. Lysozyme (400 μL, 10 mg mL⁻¹, Sigma-Aldrich, USA) was added to each sample followed by beat beating with a FastPrep instrument (6 m s⁻¹, 30 s, MP Biomedicals, Irvine, CA, USA) in 2 mL tubes containing glass beads (0.5 mm diameter, BioSpec Products, Inc., Bartlesville, OK, USA). RNA was further extracted using the RneasyMini Kit (Qiagen, Hilden, Germany)

following the manufacturer's recommendations. Quality and quantity of the extracted RNA were measured with a Nanodrop instrument (Thermo Fisher, Waltham, MA, USA). DNA was further digested with DNase I (Thermo Fisher, Waltham, MA, USA) and after the enzyme reaction cleaned using Monarch RNA Cleanup Kit (New England Biolabs, Ipswich, MA, USA). The RNA quality was determined using the Agilent Bioanalyzer 2100 Pico kit (Agilent Technologies, Santa Clara, CA, USA) and the QuantiFluor RNA kit (Quantus, Promega, Madison, WI, USA).

Sequencing

Libraries were prepared and sequencing was done at Novogene, Cambridge. Library was prepared using 1 µg RNA input and the Illumina Ribo-Zero Plus rRNA Depletion Kit (Illumina, San Diego, CA, USA) followed by NEBNext[®] UltraTM RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) following the manufacturer's recommendation. Quality assessment was done with Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Libraries were clustered using the PE Cluster Kit cBot-HS (Illumina, San Diego, CA, USA) and were further sequenced on an Illumina NovaSeq 6000.

Sequencing data analysis

Raw data processing was done at Novogene Cambridge. Raw reads were processed using fastp (Chen et al. 2018) and cleaned reads were mapped against the reference genomes using Bowtie2 (Langemead and Salzberg 2012, reference genomes: previously published genome of *P. aromaticivorans* AR20-38 (Poyntner et al. 2020; NCBI, PRJNA624061) and genome of *P. phytofirmans* (NCBI, PRJNA17463)). FeatureCounts (Liao et al. 2014) was used for counting the reads followed by calculation of fragments per kilobase of transcript per million mapped reads (FPKM). The differential gene expression analysis was performed using DESeq2 R package (Love et al. 2014). Genes with resulting *p*-values < 0.05 were assigned as differentially expressed. Gene length biases were corrected using ClusterProfilerR (Yu et al. 2012) and the gene Ontology (GO) enrichment (Ashburner et al. 2000) and Kyoto encyclopedia of genes and genomes (KEGG, Kanehisa and Goto 2000) pathway analysis were performed. Data was sorted and duplicated reads were marked using Picard (<http://broadinstitute.github.io/picard> accessed 01.09.2021) and Samtools (Li et al. 2009). Further analysis was done using R studio version 4.1.3 (R Core Team 2017) using the package ggplot (Wickham 2016). Inkscape (Inkscape Project 2020) was used for graph creation.

HPLC analysis

FA and VA were quantified with HPLC analysis as described previously (Margesin et al. 2021) in reducing intervals (28–8 h intervals, Additional file 1: Table S1). In short: after centrifugation (10 min, 20 000 × *g*) to remove larger particles the supernatants were frozen (− 20 °C) and at least 0.7 mL of the supernatant was filtered (0.2 µm RC filter) for HPLC measurement. The measurement was performed at 70 °C using a Shimadzu Prominence system equipped with a RFQ Fast Acid column (50 × 7.8 mm, Phenomenex, Torrance, CA, Germany) and a mobile phase of 5 mM sulfuric acid as described previously (Wagner et al. 2017). A UV detection at 220 nm combined with a crosscheck at 270 nm was applied. The calibration was performed via injection of 1, 5 and 10 mM FA and VA external standards.

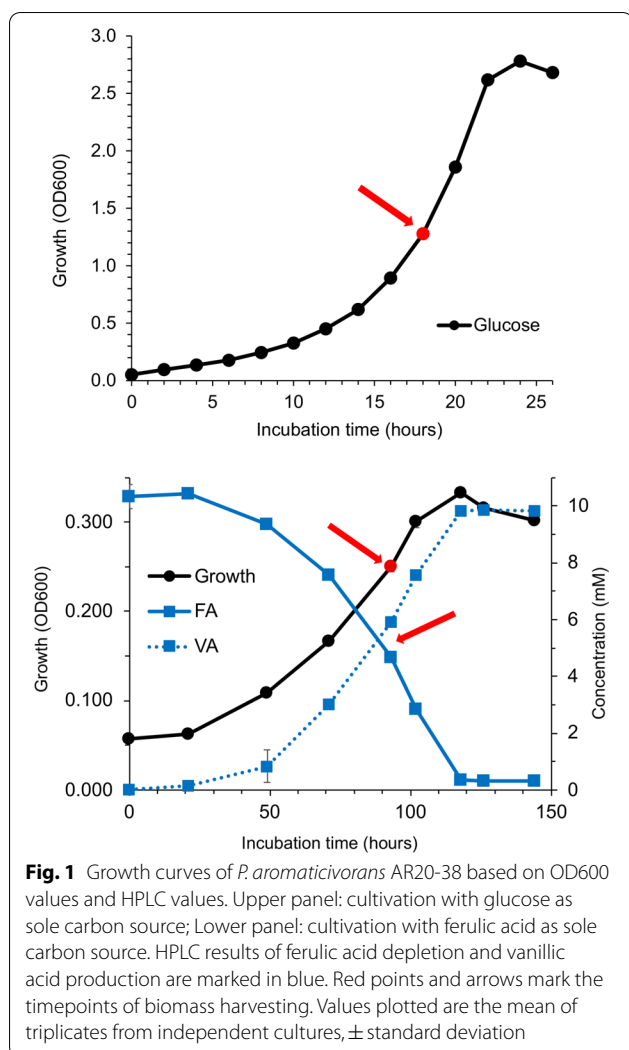
The rate constant of decline (*k*), DT50 was calculated using Computer Assisted Kinetic Evaluation (CAKE) software (available online with public free access, <https://cake-kinetics.org/>, accessed: 21.04.2022) using a convergence tolerance of 1×10^{-5} , 100 max. iterations and iteratively reweighted least squares (max. reweightings: 200, error variance tolerance: 1×10^{10}) and a simple first order fit.

Results

The strain *P. aromaticivorans* AR20-38 exhibited very good bioconversion capability of FA to VA at low and moderate temperatures (10–30 °C) (Berger et al. 2021; Margesin et al. 2021) but the gene expression patterns during the bioconversion of FA to VA remained unclear. To close this knowledge gap, two experiments were set up in the present study: the strain was cultivated with (i) glucose (G1-3) and (ii) FA (FA1-3) as sole carbon source in triplicates at 20 °C. In the exponential growth phase (after 18 h of cultivation for glucose and after 96 h for FA, Fig. 1) the cells were harvested, and RNA was extracted and sequenced. The strain exhibited a rate constant of decline for FA (*k*) of 0.63 (σ : 0.0031; r^2 : 0.8) and a DT50 of 55.5 h.

Transcriptome sequencing

The cDNA sequencing of the samples yielded in 15.3–10.8 million raw reads (Additional file 1: Table S2). After removing reads with adaptor sequence contamination, uncertain nucleotides and low-quality nucleotides 15.2–10.8 million clean reads were obtained. The base error rate of all samples was 0.02%. The mapping resulted in of 12 to 8 million uniquely mapped reads. In the principal component analysis (PCA) analysis (Additional file 1: Fig. S1) the three biological replicates from three independent cultures of the two conditions (G, FA) clustered well together, and the sequenced samples G1-3 and FA1-3



were clearly separated. Therein, G1-3 replicates were very similar and FA1-2 differed slightly from FA3.

In total 5049 genes were expressed, 306 were only expressed in the samples with FA and 59 solely with glucose as sole carbon source. 1653 genes were upregulated in the FA samples compared to 1682 downregulated genes.

Transcriptional response to ferulic acid

The highest differentially expressed genes clustered well within the triplicates and transcripts reflect the activities associated with FA as sole carbon source (Fig. 2). Genes coding for enzymes involved in bioconversion of FA were within the ten most highly upregulated genes ($p < 0.05$, Additional file 1: Table S3) when *P. aromaticivorans* was cultivated with FA as sole carbon source. Three genes directly involved in the bioconversion were determined: 4-coumarate-CoA ligase (RS20035, $\log_2FC:89$),

hydroxycinnamoyl-CoA hydratase lyase (RS20025, $\log_2FC: 9.6$) and vanillin dehydrogenase (RS20030, $\log_2FC:9.1$).

Mono(2-hydroxyethyl) terephthalate hydrolase (RS20005, $\log_2FC:11.36$) was within the ten most upregulated genes. This enzyme was previously described in *Ideonella sakaiensis* (Yoshida et al. 2016) and hydrolyzes polyethylene terephthalate (PET)-derived mono(2-hydroxyethyl) terephthalate to terephthalic acid and ethylene glycol (Knott et al. 2020). The second enzyme responsible for PET degradation in *I. sakaiensis*, the PETase, was not detected in *P. aromaticivorans*.

The 3-(3-hydroxy-phenyl)propionate transporter was highly upregulated (RS20015, $\log_2FC:11.7$) as well as the outer membrane porin protein BP0840 (RS20010, $\log_2FC: 11.5$), important for transports.

Uroporphyrinogen-III C-methyltransferase (RS09835, $\log_2FC:11.5$) was upregulated and is involved in the cobalamin biosynthesis. Similarly, in a transcriptome study on lignin valorisation (Zhu et al. 2021) cobalt-precorrin-3B C(17)-methyltransferase, which is involved in the adenosyl cobalamin biosynthesis, was upregulated.

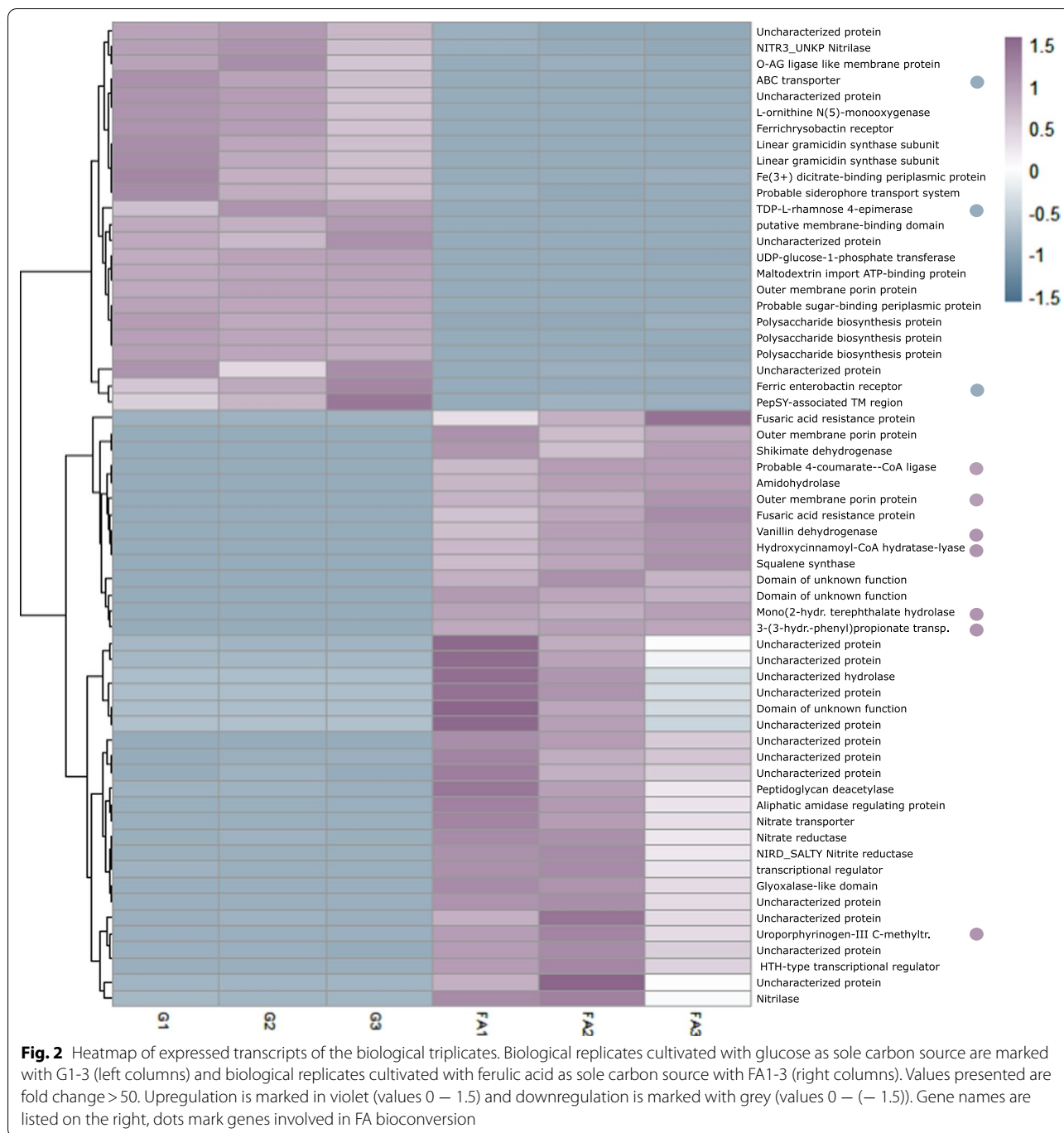
The NIRD_SALTY nitrite reductase (NADH) small subunit (RS09845/ RS09840, $\log_2FC: 11.3/9.6$) and MFS_1 nitrate transporter (RS09855, $\log_2FC: 7.17$) were highly upregulated in *P. aromaticivorans* cultivated with FA as sole carbon source.

In other bacterial strains the bioconversion of VA to protocatechuic acid is catalyzed by the enzyme vanillate O-demethylase consisting of two subunits: VanA oxygenase and VanB oxidoreductase. Here, the gene for VanA was upregulated (RS34940, $\log_2FC: 1.9$) whereas VanB was downregulated (RS10660, $\log_2FC: - 1.1$).

In *P. aromaticivorans* cultivated with FA, the highest downregulated gene was dTDP-L-rhamnose 4-epimerase (RS31050, $\log_2FC: - 9.4$), involved in the interconversion of dTDP-rhamnose to dTDP-6d talose. Additionally, the ABC transporter for syringomycin transport (RS20255, $\log_2FC: - 8.7$) was downregulated in *P. aromaticivorans* in presence of FA.

P. aromaticivorans downregulated the ferric enterobactin receptor (RS22965, $\log_2FC: - 8.3$) in presence of FA compared to glucose as sole carbon source. The cultivation medium for both conditions contained 0.1 mg l^{-1} iron respectively.

Additional iron related genes were differently expressed, three down and three slightly upregulated: the ferric iron reductase (RS20240, $\log_2FC: - 4.7$), ferrous iron permease (RS06920, $\log_2FC: - 4.7$), iron sulfur cluster assembly protein CyaY (RS17920, $\log_2FC: - 0.8$) were downregulated and the probable iron transporter (RS09245, $\log_2FC:0.6$), iron-sulfur cluster carrier protein (RS04965, $\log_2FC:0.4$), iron-sulfur cluster assembly



scaffold protein (RS12775, \log_2FC : 0.03) were slightly upregulated.

Transporters and transcriptional factors

The data was screened based on transcriptional factors reported by Tropel and van der Meer (2004) and their importance for the degradation pathways of aromatic compounds. *P. aromaticivorans* differentially expressed

134 genes in the LysR group, 47 up and 87 down. Within the group, CatR was upregulated (RS10680, \log_2FC : 0.1), a transcriptional factor involved in the catechol pathway. Further, BenM (RS07845, \log_2FC : 1.0), involved in the benzoate (catechol) pathway and two genes of NahR, both involved in the naphthalene and salicylate pathway (RS30310/ RS21410, \log_2FC : – 0.7/– 0.1), were down-regulated. Twenty-one IclR genes were differentially

expressed, eight down and 13 up. Within the IclR group, PcaU (RS18385, log₂FC: 1.9) and two genes coding for PCAR (RS13595/RS29425, log₂FC: -1.3/0.2) were differentially expressed.

Twenty-three MarR genes (9 up, 14 down) and 26 TetR genes (14 down, 12 up) were differentially expressed. These transcriptional factors regulate aromatic compounds transporters.

In the transcriptome of *P. aromaticivorans* 336 transporters were differentially expressed. Of these transporters, 161 are categorized as ATP-binding cassette (ABC) transporters and as 96 major facilitator superfamily (MFS) transporters. The ABC transporter spermidine/putrescine was highly upregulated (RS26120, log₂FC: 5.6). This transporter is responsible for transporting polyamines which are important for cell proliferation and for ion homeostasis. The upregulated sugar transporter (RS26045, log₂FC: 5.4) belongs to the ABC group which was reported in *Thermobifida fusca* (Adav et al. 2012) cultivated on different lignocellulosic materials e.g. hay, saw dust, wood chip, un-dried plant biomass. The shikimate transporter (RS20435, log₂FC: 5.2) was upregulated, transporting shikimate, an intermediate product in the aromatic compound biosynthetic pathway (Kubota et al.

2015). The second most important group of transporters are the MFS group. These transporters are reported to be involved in FA transport in other bacteria (Winkler and Kao 2011). Two MFS transporters were within the ten highest differentially expressed genes: 3-(3-hydroxyphenyl) propionate transporter and MFS_1 nitrate transporter (see Sect. [Transcriptional response to ferulic acid](#)).

P. aromaticivorans upregulated the maltodextrin import in the experiment with glucose compared to FA, used to import glucose (RS05040, log₂FC: -5.9). Further, a probable siderophore transport system (RS20230, -log₂FC: -5.7) was downregulated in the FA experiment.

Enrichment KEGG Terms

In the cultivation with FA as sole carbon source, *P. aromaticivorans* upregulated genes of the KEGG term (Fig. 3, Additional file 1: Table S5) microbial metabolism in diverse environments (bpy01120) and benzoate degradation (bpy00362, Fig. 3, Additional file 1: Table S5). Both terms contain pathways involving ring cleaving enzymes. In presence of FA *P. aromaticivorans* upregulated the term starch and sucrose metabolism (bpy00500) to increase the carbohydrate metabolism.

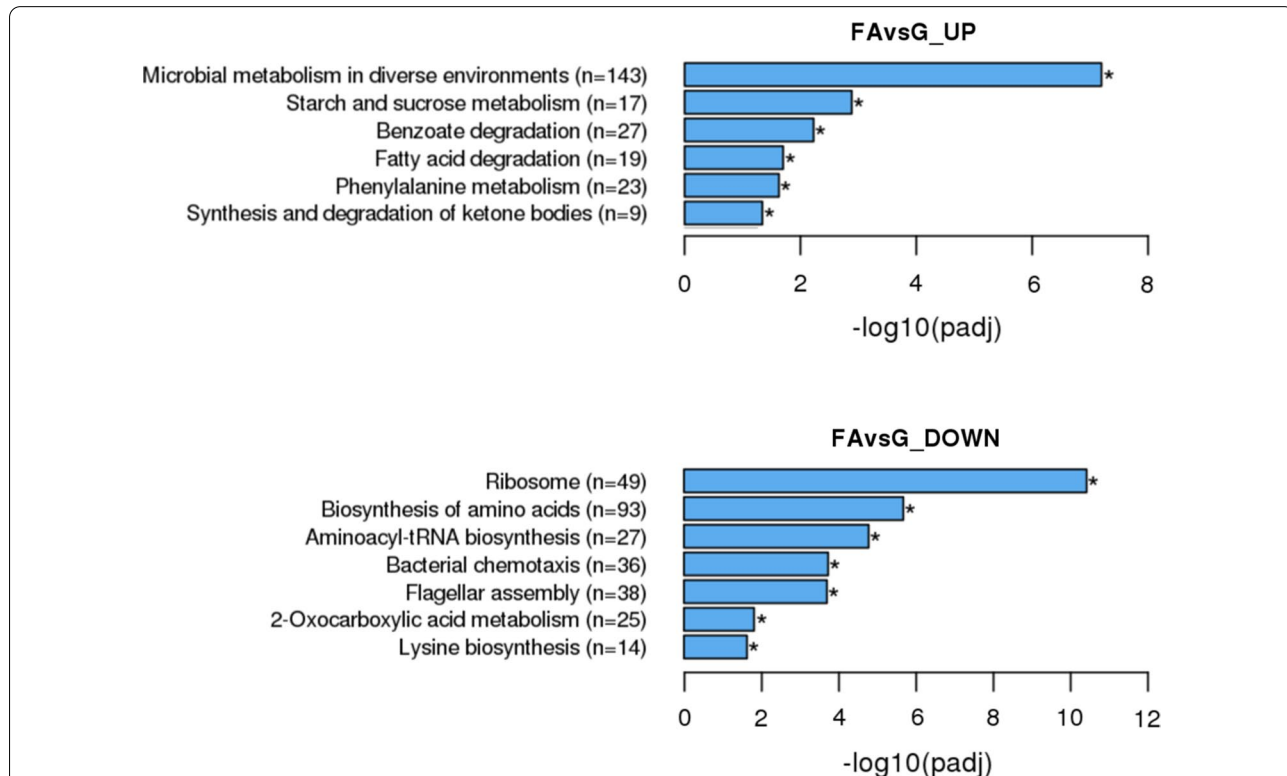


Fig. 3 Significantly up- and downregulated KEGG terms. The upper panel represents the upregulated terms and the lower panel the downregulated terms of *P. aromaticivorans* cultivated with ferulic acid in comparison to glucose as sole carbon source. Bars represent the -log₁₀ adjusted *p*-value

The upregulated term fatty acid degradation (bpy00071) contains fatty acid oxidation enzymes such as enoyl-SCoA hydratase. The term phenylalanine metabolism (bpy00360) was upregulated comprising the modules phenylacetate degradation and trans-cinnamate degradation. In both modules, enzymes involved lignin and FA biodegradation are found. Nine genes fit in the term synthesis and degradation of ketone bodies (bpy00072).

KEGG terms related to growth were upregulated in the cultivation of *P. aromaticivorans* with glucose as sole carbon source whereas in the cultivation with FA as sole carbon source these terms were downregulated: Ribosome (bpy03010), biosynthesis of amino acids (bpy01230) and aminoacyl-tRNA biosynthesis (bpy00970). Further bacterial chemotaxis (bpy02030), flagellar assembly (bpy02040), 2-oxocarboxylic acid metabolism (bpy01210) and lysine biosynthesis (bpy00300) were downregulated in presence of FA.

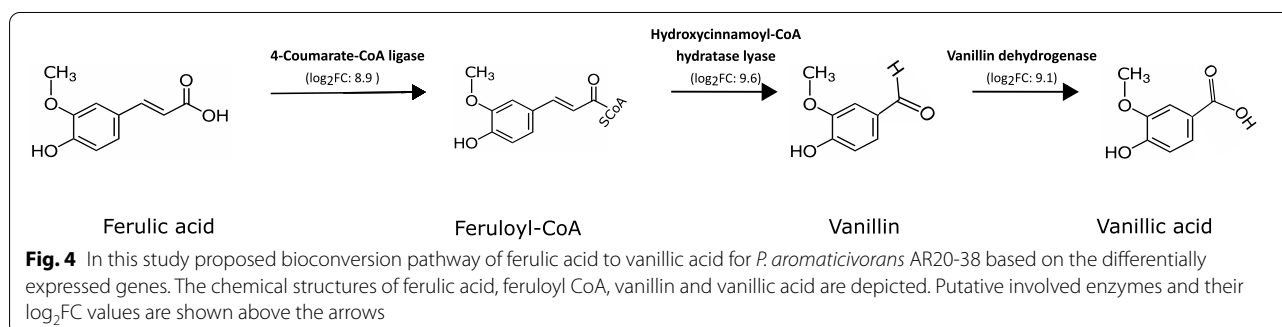
Discussion

Based on the strongly differentially expressed genes in the transcriptome of *P. aromaticivorans* during bioconversion of FA to VA in this study, the pathway depicted in Fig. 4 is proposed. 4-coumarate-CoA ligase is a known enzyme in FA metabolism responsible for the step FA to feruloyl-CoA and was previously reported in *Streptomyces coelicolor* (Kaneko et al. 2003) and in plants (Wohl and Petersen 2020). Subsequently, hydroxycinnamoyl-CoA hydratase lyase converts feruloyl-CoA to vanillin, which was previously reported in *Pseudomonas fluorescens* (Bennett et al. 2008). Vanillin dehydrogenase is a known enzyme for Vanillin metabolization to VA. *P. aromaticivorans* was not able to further degrade VA through the protocatechuate pathway and bioaccumulated VA (Margesin et al. 2021). In other bacteria, this step is catalyzed by the enzyme vanillate *O*-demethylase encoded by the genes VanA and VanB, which were only slightly regulated in *P. aromaticivorans*, VanB was downregulated. In contrast, PcaU was slightly

upregulated, a transcriptional factor reported to play a role in the protocatechuate pathway. *P. aromaticivorans* harbors the gene for protocatechuate-3,4-dioxygenase (Poyntner et al. 2021) but the gene was not upregulated during FA bioconversion (Additional file 1: Table S3). This indicates that PcaU is involved in the previous steps of FA to VA bioconversion.

The upregulated KEGG terms can be related to FA bioconversion. The main upregulated KEGG terms involve pathways with ring cleaving enzymes, which are important for the bioconversion of FA to VA. For example, the KEGG term microbial metabolism in diverse environments comprises various pathways including carbohydrate metabolism, energy metabolism, sulfur- and amino acid metabolism, as well as xenobiotic degradation. Further, the upregulated KEGG term benzoate degradation can be related to the frequently reported ability to degrade benzoate in *Paraburkholderia* strains (Donoso et al. 2016; Herpell et al. 2021; Vanwijnsberghe et al. 2021). The upregulated term fatty acid degradation contains fatty acid oxidation enzymes such as enoyl-SCoA hydratase. These are reported to be involved in the degradation of FA (Gasson et al. 1998). The upregulated KEGG term phenylalanine metabolism could be linked to osmotic stress response as previously reported in *Burkholderia cenocepacia* (Behrends et al. 2011) but also comprises enzymes involved lignin and FA biodegradation.

The highly regulated mono(2-hydroxyethyl) terephthalate hydrolase was previously reported in *I. sakaiensis* (Yoshida et al. 2016) during PET degradation. *I. sakaiensis* did not show any activity against ethyl ferulate (Yoshida et al. 2016). Although the gene coding for PETase is missing the genome and transcriptome of *P. aromaticivorans*, the strain showed growth in presence of bisphenol A, a chemical plasticizer, in previous tests (data not shown). A closely related species, *B. xenovorans*, was reported to be an effective polychlorinated biphenyl degrader (Chain et al. 2006). Additionally, the KEGG term lysine biosynthesis was regulated, which was detected in *B. xenovorans* (Parnell et al. 2006) in



presence of polychlorinated biphenyls. Therefore, *P. aromaticivorans* might be a good candidate for plastic but also xenobiotic degradation. In future studies, the involvement of mono(2-hydroxyethyl) terephthalate hydrolase in FA bioconversion needs to be studied in detail which could be also important for other FA bioconverting organisms.

Uroporphyrinogen-III C-methyltransferase was highly regulated, which is involved in cobalamin biosynthesis. Similarly, cobalt-precorrin-3B C(17)-methyltransferase was upregulated in a transcriptome study on lignin valorisation (Zhu et al. 2021). This enzyme is involved in the adenosyl cobalamin biosynthesis. Cobalamin produced by *Rhizobium* isolates from forest soils were shown to enhance methane oxidation of a methanotroph (Iguchi et al. 2011). A similar mechanism might play a role in *P. aromaticivorans*.

The most regulated transporters in the transcriptome of *P. aromaticivorans* during bioconversion of FA were ABC transporters and MFS transporters. ABC transporters are reported in *Enterobacter lignolyticus* (DeAngelis et al. 2013), *Clostridium beijerinckii* (Lee et al. 2015; Zhang and Ezeji 2013) and *Lactobacillus brevis* (Winkler and Kao 2011) to be involved in the transport of lignin-derived aromatic substances such as FA. The regulated ABC sugar transporter and spermidine/putrescine might be used for nutrient acquisition in *P. aromaticivorans* during FA bioconversion.

Within the MFS group, the MFS_1 nitrate transporter was highly regulated, indicating utilization of nitrate. This is in contrast to previously reported results detecting no nitrate reduction capability in the strain *P. aromaticivorans* BNT5^T (Lee and Jeon 2018). Additionally, the NIRD_SALTY nitrite reductase small subunit was highly upregulated. It was previously shown in denitrifying bacteria that the addition of nitrate improved the biodegradation processes of toluene as nitrate can be used as an alternative electron acceptor (Leahy and Olsen 1997).

Another putative transport mechanisms during FA bioconversion is the upregulated 3-(3-hydroxy-phenyl) propionate transporter, which also belongs to the MFS group. Phenylpropionate and its hydroxylated derivatives are degradation products of lignin and were shown to be degraded by various bacteria (Arai et al. 1999; Barnes et al. 1997; Burlingame and Chapman 1983; Dagley et al. 1965; Pérez-Pantoja et al. 2008; Xu et al. 2013). The regulated transcription factors MarR and TetR are known regulators of aromatic compounds transporters for degradation pathways including HpcR involved in the hydroxycinnamate pathway (Tropel and van der Meer 2004). Thus, this indicates an involvement of these transcription factors for transporting mechanisms in *P. aromaticivorans* during bioconversion of FA. Although

many transcriptional factors are annotated in the genome of *P. aromaticivorans*, only a few of the highly differentially expressed transcriptional factors could be related to previously reported involvement in aromatic compound degradation. The targets of the differentially expressed transcription factors in this study still need to be studied.

The enzymes for catabolizing FA might be transported through the highly regulated outer membrane porin protein. This is in line with the upregulation of the GO term transmembrane transport (Additional file 1: Table S4). *P. aromaticivorans* might use a mechanism based on outer membrane vesicles (OMVs) as reported by Salvachúa et al. (2020) in *P. putida*. OMVs in *P. putida* are used to catabolize lignin-derived substances which are transported via porin proteins. The utilization of OMVs has also been suggested as a synthetic biology tool and the OMV model harbors several potential biotechnological applications (Salvachúa et al. 2020).

Several downregulated genes during bioconversion of FA indicate plant interaction capabilities of *P. aromaticivorans*. *Burkholderia* species were previously reported to act as plant promoting (Herpell et al. 2021; Donoso et al. 2016). The ABC transporter for syringomycin was downregulated, reported as a potential plant virulence factor in *B. thailandensis* (Kovacs-Simon et al. 2019). The downregulation of the KEGG term chemotaxis indicates potential plant interaction capabilities. Sheibani-Tezerji et al. (2015) reported chemotaxis in the transcriptome of the endophyte *B. phytophormans* and Balsanelli et al. (2016) showed that chemotaxis plays an important role in initial plant contact. Further, due to the higher growth rate with glucose compared to FA, *P. aromaticivorans* needed more cell wall components and therefore might upregulated dTDP-L-rhamnose 4-epimerase in presence of glucose. This epimerase is involved in the production of L-rhamnose, a polysaccharide component of pathogenic or plant associated bacteria (Graninger et al. 1999). Additionally, the epimerase is involved in the interconversion to dTDP-6-deoxy-D-talose, which was shown in *B. thailandensis* (Yoo et al. 2011) to serve as building block for O-antigenic polysaccharide biosynthesis. The O-antigenic polysaccharide is a reported virulence factor in *Burkholderia pseudomallei* (DeShazer et al. 1998). In presence of glucose, enterobactin, an iron siderophore, ferric iron reductase, ferrous iron permease and probable siderophore transport system were upregulated. This indicates the importance of iron during high growth rates but not during bioconversion of FA. Catechol-like siderophore production was previously reported from rhizospheric bacteria (Joshi et al. 2006) which gives them advantage to sequester iron from soil.

The downregulated KEGG terms related to growth fit well with the higher growth rate with glucose as sole

carbon source in comparison to FA. The regulated term 2-oxocarboxylic acid metabolism might be involved in the glucose metabolism in the cultivation of *P. aromaticivorans* with glucose as sole carbon source. The upregulated KEGG term synthesis and degradation of ketone bodies cannot be related to any *P. aromaticivorans* metabolism.

In conclusion, the transcriptional profile of *P. aromaticivorans* during bioconversion of FA to VA elucidated the expressed genes involved in the bioconversion process. These genes were clustered within the ten most highly differentially expressed genes. These enzymes and the transcripts known from aromatic compound degradation and synthetic polymer degradation offer new gene targets for bioconversion optimization and metabolic engineering. In addition, the strong expression of an outer membrane porin was detected, indicating towards the occurrence of a recently proposed outer membrane vesicle mechanisms for bacterial lignin catabolism (Salvachúa et al. 2020). To confirm this hypothesis, further studies on exosomes using e.g. exoproteome experiments are needed. These could offer a number of new applications for synthetic biology that may even lead to novel biotechnological applications.

Overall, the strain AR20-38 is a promising candidate not only for FA bioconversion applications but also for xenobiotic and synthetic polymer degradation, especially in moderate and cold temperature environments.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13568-022-01487-7>.

Additional file 1: Figure S1. PCA analysis. The RNA-sequencing results of the 3 biological replicates cultivated with glucose (G1-3) or ferulic acid (FA1-3) are depicted. **Table S1.** OD600 and HPLC values. **Table S2.** Sequencing statistics. **Table S3.** Gene expression values. **Table S4.** Regulated GO terms. **Table S5.** Regulated KEGG terms.

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Author contributions

RM and CP planned the experiments. TL and CP performed the cultivations and RNA extractions, TL prepared the HPLC samples and measured the OD600 values, AOW established and performed the HPLC measurements and AOW, TL and RM analyzed the HPLC and OD600 data. CP analyzed the transcriptome data and wrote the manuscript. All authors contributed to the manuscript and agreed with the publication. RM and AOW financially supported analysis and publication. All authors read and approved the final manuscript.

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Data availability

Rawdata (Fastq files) of the sequenced samples were deposited in the NCBI Sequencing Read Archive under the numbers SRR18913740, SRR18913739, SRR18913740, SRR18913578, SRR18913579 and SRR18913580 within the Bioproject PRJNA624061.

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no conflict of interest.

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References

- Adav SS, Cheow ESH, Ravindran A, Dutta B, Sze SK (2012) Label free quantitative proteomic analysis of secretome by *Thermobifida fusca* on different lignocellulosic biomass. *J Proteomics* 75:3694–3706. <https://doi.org/10.1016/j.jprot.2012.04.031>
- Anastas P, Eghbali N (2010) Green chemistry: principles and practice. *Chem Soc Rev* 39:301–312. <https://doi.org/10.1039/B918763B>
- Arai H, Yamamoto T, Ohishi T, Shimizu T, Nakata T, Kudo TY (1999) Genetic organization and characteristics of the 3-(3-hydroxyphenyl)propionic acid degradation pathway of *Comamonas testosteroni* TA441. *Microbiology* 145:2813–2820. <https://doi.org/10.1099/00221287-145-10-2813>
- Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000) Gene ontology: tool for the unification of biology. *Nat Genet* 25:25–29. <https://doi.org/10.1038/75556>
- Balsanelli E, Tadra-Sfeir MZ, Faoro H, Pankievicz VC, de Baura VA, Pedrosa FO, de Souza EM, Dixon R, Monteiro RA (2016) Molecular adaptations of *Herbaspirillum seropedicae* during colonization of the maize rhizosphere. *Environ Microbiol* 18:2343–2356. <https://doi.org/10.1111/1462-2920.12887>
- Barnes MR, Duetz WA, Williams PA (1997) A 3-(3-hydroxyphenyl)propionic acid catabolic pathway in *Rhodococcus globerulus* PWD1: cloning and characterization of the hpp operon. *J Bacteriol* 179:6145–6153. <https://doi.org/10.1128/jb.179.19.6145-6153.1997>
- Behrends V, Jg B, Williams Hd (2011) Differences in strategies to combat osmotic stress in *Burkholderia cenocepacia* elucidated by NMR-based metabolic profiling. *Lett Appl Microbiol* 52:619–625. <https://doi.org/10.1111/j.1472-765X.2011.03050.x>
- Bennett JP, Bertin L, Moulton B, Fairlamb IJS, Brzozowski AM, Walton NJ, Grogan G (2008) A ternary complex of hydroxycinnamoyl-CoA hydratase-lyase (HCHL) with acetyl-CoA and vanillin gives insights into substrate specificity and mechanism. *Biochem* 414:281–289. <https://doi.org/10.1042/BJ20080714>
- Berger T, Poyntner C, Margesin R (2021) Culturable bacteria from an Alpine coniferous forest site: biodegradation potential of organic polymers and pollutants. *Folia Microbiol* 66:87–98. <https://doi.org/10.1007/s12223-020-00825-1>
- Burlingame R, Chapman PJ (1983) Catabolism of phenylpropionic acid and its 3-hydroxyderivative by *Escherichia coli*. *J Bacteriol* 155:113–121. <https://doi.org/10.1128/jb.155.1.113-121.1983>
- Bugg TD, Ahmad M, Hardiman EM, Singh R (2011) The emerging role for bacteria in lignin degradation and bio-product formation. *Curr Opin Biotechnol* 22:394–400. <https://doi.org/10.1016/j.copbio.2010.10.009>
- Cao L, Yu IKM, Liu Y, Ruan X, Tsang DCW, Hunt AJ, Ok YS, Song H, Zhang S (2018) Lignin valorization for the production of renewable chemicals:

- state-of-the-art review and future prospects. *Bioresour Technol* 269:465–475. <https://doi.org/10.1016/j.biortech.2018.08.065>
- Chain PSG, Denev VJ, Konstantinidis KT, Vergez LM, Agulló L, Reyes VL, Hauser L, Córdoba M, Gómez L, González M, Land M, Lao V, Larimer F, LiPuma JJ, Mahenthiralingam E, Malfatti SA, Marx CJ, Parnell JJ, Ramette A, Richardson P, Seeger M, Smith D, Spilker T, Sul WJ, Tsoi TV, Ulrich LE, Zhulin IB, Tiedje JM (2006) *Burkholderia xenovorans* LB400 harbors a multi-replicon, 9.73-Mbp genome shaped for versatility. *PNAS* 103:15280–15287. <https://doi.org/10.1073/pnas.0606924103>
- Chen S, Zhou Y, Chen Y, Gu J (2018) fastp: an ultra-fast all-in-one FASTQ pre-processor. *J Bioinform* 34:i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>
- Civolani C, Barghini P, Roncetti AR, Ruzzi M, Schiesser A (2000) Bioconversion of ferulic acid into vanillic acid by means of a vanillate-negative mutant of *Pseudomonas fluorescens* strain BF13. *Appl Environ Microbiol* 66:2311–2317. <https://doi.org/10.1128/AEM.66.6.2311-2317.2000>
- Dagley S, Chapman PJ, Gibson DT (1965) The metabolism of β -phenylpropionic acid by an *Achromobacter*. *Biochem J* 97:643–650. <https://doi.org/10.1042/bj0970643>
- DeAngelis K, Sharma D, Varney R, Simmons B, Isern N, Markillie LM, Nicora C, Norbeck A, Taylor R, Aldrich J, Robinson E (2013) Evidence supporting dissimilatory and assimilatory lignin degradation in *Enterobacter lignolyticus* SCF1. *Front Microbiol*. <https://doi.org/10.3389/fmicb.2013.00280>
- DeShazer D, Brett PJ, Woods DE (1998) The type II O-antigenic polysaccharide moiety of *Burkholderia pseudomallei* lipopolysaccharide is required for serum resistance and virulence. *Mol Microbiol* 30:1081–1100. <https://doi.org/10.1046/j.1365-2958.1998.01139>
- Donoso R, Leiva-Novoa P, Zúñiga A, Timmermann T, Recabarren-Gajardo G, González B (2016) Biochemical and genetic bases of indole-3-acetic acid (auxin phytohormone) degradation by the plant-growth-promoting rhizobacterium *Paraburkholderia phytofirmans* PsJN. *Appl Environ Microbiol* 83:e01991–e2016. <https://doi.org/10.1128/AEM.01991-16>
- Fuchs G, Boll M, Heider J (2011) Microbial degradation of aromatic compounds—from one strategy to four. *Nat Rev Microbiol* 9:803–816. <https://doi.org/10.1038/nrmicro2652>
- Gasson MJ, Kitamura Y, McLaughlan WR, Narbad A, Parr AJ, Parsons ELH, Payne J, Rhodes MJ, Walton NJ (1998) Metabolism of ferulic acid to vanillin: a bacterial gene of the enoyl-SCO-A hydratase/isomerase superfamily encodes an enzyme for the hydration and cleavage of hydroxycinnamic acid SCO-A thioester. *J Biol Chem* 273:4163–4170. <https://doi.org/10.1074/jbc.273.7.4163>
- Graf N, Altenbuchner J (2014) Genetic engineering of *Pseudomonas putida* KT2440 for rapid and high-yield production of vanillin from ferulic acid. *Appl Microbiol Biotechnol* 98:137–149. <https://doi.org/10.1007/s00253-013-5303-1>
- Graninger M, Nidetzky B, Heinrichs DE, Whitfield C, Messner P (1999) Characterization of dTDP-4-dehydrorhamnose 3,5-epimerase and dTDP-4-dehydrorhamnose reductase, required for dTDP-L-rhamnose biosynthesis in *Salmonella enterica* serovar Typhimurium LT2. *J Biol Chem* 275:25069–25077. <https://doi.org/10.1074/jbc.274.35.25069>
- Herpell JB, Vanwijnsberghe S, Peeters C, Schindler F, Fragner L, Bejtović M, Weckwerth W, Vandamme P (2021) *Paraburkholderia dioscraea* sp. nov., a novel plant associated growth promotor. *Int J Syst Evol Microbiol* 71:004969. <https://doi.org/10.1099/ijsem.0.004969>
- Iguchi H, Yurimoto H, Sakai Y (2011) Stimulation of methanotrophic growth in cocultures by cobalamin excreted by *Rhizobia*. *Appl Environ Microbiol*. <https://doi.org/10.1128/AEM.05834-11>
- Inkscape Project (2020) Inkscape. Retrieved from <https://inkscape.org>. Accessed May 2022.
- Joshi F, Archana G, Desai A (2006) Siderophore cross-utilization amongst rhizospheric bacteria and the role of their differential affinities for Fe³⁺ on growth stimulation under iron-limited conditions. *Curr Microbiol* 53:141. <https://doi.org/10.1007/s00284-005-0400-8>
- Kamimura N, Takahashi K, Mori K, Araki T, Fujita M, Higuchi Y, Masai E (2017) Bacterial catabolism of lignin-derived aromatics: New findings in a recent decade: Update on bacterial lignin catabolism. *Environ Microbiol Rep* 9:679–705. <https://doi.org/10.1111/1758-2229.12597>
- Kanehisa M, Goto S (2000) KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 28:27–30. <https://doi.org/10.1093/nar/28.1.27>
- Kaneko M, Ohnishi Y, Horinouchi S (2003) Cinnamate: coenzyme A ligase from the filamentous bacterium *Streptomyces coelicolor* A3(2). *J Bacteriol* 185:20–27. <https://doi.org/10.1128/JB.185.1.20-27.2003>
- Knott BC, Erickson E, Allen MD, Gado JE, Graham R, Kearns FL, Pardo I, Topuzlu E, Anderson JJ, Austin HP, Dominick G, Johnson CW, Rorrer NA, Szostkiewicz CJ, Copié V, Payne CM, Woodcock HL, Donohoe BS, Beckham GT, McGeehan JE (2020) Characterization and engineering of a two-enzyme system for plastics depolymerization. *PNAS* 117:25476–25485. <https://doi.org/10.1073/pnas.2006753117>
- Kovacs-Simon A, Hemsley CM, Scott AE, Prior JL, Titball RW (2019) *Burkholderia thailandensis* strain E555 is a surrogate for the investigation of *Burkholderia pseudomallei* replication and survival in macrophages. *BMC Microbiol* 19:97. <https://doi.org/10.1186/s12866-019-1469-8>
- Kubota T, Tanaka Y, Takemoto N, Hiraga K, Yukawa H, Inui M (2015) Identification and expression analysis of a gene encoding a shikimate transporter of *Corynebacterium glutamicum*. *Microbiology* 161:254–263. <https://doi.org/10.1099/mic.0.083733-0>
- Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9:357–359. <https://doi.org/10.1038/nmeth.1923>
- Leahy JG, Olsen RH (1997) Kinetics of toluene degradation by toluene-oxidizing bacteria as a function of oxygen concentration, and the effect of nitrate. *FEMS Microbiol Ecol* 23:23–30. <https://doi.org/10.1111/j.1574-6941.1997.tb00387>
- Lee Y, Jeon CO (2018) *Paraburkholderia aromaticivorans* sp. Nov., an aromatic hydrocarbon-degrading bacterium, isolated from gasoline-contaminated soil. *Int J Syst Evol Microbiol*. <https://doi.org/10.1099/ijsem.0.002661>
- Lee S, Lee JH, Mitchell RJ (2015) Analysis of *Clostridium beijerinckii* NCIMB 8052's transcriptional response to ferulic acid and its application to enhance the strain tolerance. *Biotechnol Biofuels* 8:68. <https://doi.org/10.1186/s13068-015-0252-9>
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome project data processing subgroup (2009) The sequence alignment/map format and SAMtools. *Bioinformatics* 25:2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>
- Liao Y, Smyth GK, Shi W (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 30:923–930. <https://doi.org/10.1093/bioinformatics/btt656>
- Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15:550. <https://doi.org/10.1186/s13059-014-0550-8>
- Margesin R, Schinner F (1997) Bioremediation of diesel-oil-contaminated alpine soils at low temperatures. *Appl Microbiol Biotechnol* 47:462–468. <https://doi.org/10.1007/s002530050957>
- Margesin R, Volgger G, Wagner AO, Zhang D, Poyntner C (2021) Biodegradation of lignin monomers and bioconversion of ferulic acid to vanillic acid by *Paraburkholderia aromaticivorans* AR20-38 isolated from Alpine forest soil. *Appl Microbiol Biotechnol* 105:2967–2977. <https://doi.org/10.1007/s00253-021-11215-z>
- Mori Y, Noda S, Shirai T, Kondo A (2021) Direct 1,3-butadiene biosynthesis in *Escherichia coli* via a tailored ferulic acid decarboxylase mutant. *Nat Commun* 12:2195. <https://doi.org/10.1038/s41467-021-22504-6>
- Parnell JJ, Park J, Denev V, Tsoi T, Hashsham S, Quensen J, Tiedje JM (2006) Coping with polychlorinated biphenyl (PCB) toxicity: Physiological and genome-wide responses of *Burkholderia xenovorans* LB400 to PCB-mediated stress. *Appl Environ Microbiol* 72:6607–6614. <https://doi.org/10.1128/AEM.01129-06>
- Pérez-Pantoja D, De la Iglesia R, Pieper DH, González B (2008) Metabolic reconstruction of aromatic compounds degradation from the genome of the amazing pollutant-degrading bacterium *Cupriavidus necator* JMP134. *FEMS Microbiol Rev* 32:736–794. <https://doi.org/10.1111/j.1574-6976.2008.00122.x>
- Poyntner C, Zhang D, Margesin R (2020) Draft genome sequence of the bacterium *Paraburkholderia aromaticivorans* AR20-38, a Gram-negative, cold-adapted degrader of aromatic compounds. *Microbiol Resour Announc* 9:e00463–e520. <https://doi.org/10.1128/MRA.00463-20>
- Poyntner C, Kutzner A, Margesin R (2021) Biodegradation potential and putative catabolic genes of culturable bacteria from an Alpine deciduous forest site. *Microorganisms* 9:1920. <https://doi.org/10.3390/microorgan91920>

- R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>. Accessed May 2022
- Salvachúa D, Werner AZ, Pardo I, Michalska M, Black BA, Donohoe BS, Haugen SJ, Katahira R, Notonier S, Ramirez KJ, Amore A, Purvine SO, Zink EM, Abraham PE, Giannone RJ, Poudel S, Laible PD, Hettich RL, Beckham GT (2020) Outer membrane vesicles catabolize lignin-derived aromatic compounds in *Pseudomonas putida* KT2440. *PNAS* 117:9302–9310. <https://doi.org/10.1073/pnas.1921073117>
- Sheibani-Tezerji R, Rattei T, Sessitsch A, Trognitz F, Mitter B (2015) Transcriptome profiling of the endophyte *Burkholderia phytofirmans* PsJN indicates sensing of the plant environment and drought stress. *Mbio* 6:e00621–e715. <https://doi.org/10.1128/mBio.00621-15>
- Tropel D, van der Meer JR (2004) Bacterial transcriptional regulators for degradation pathways of aromatic compounds. *Microbiol Mol Biol Rev* 68:474–500. <https://doi.org/10.1128/MMBR.68.3.474-500.2004>
- Upadhyay P, Singh NK, Tupe R, Odenath A, Lali A (2020) Biotransformation of corn bran derived ferulic acid to vanillic acid using engineered *Pseudomonas putida* KT2440. *Prep Biochem Biotechnol* 50:341–348. <https://doi.org/10.1080/10826068.2019.1697935>
- Vanwijnsberghe S, Peeters C, De Ridder E, Dumolin C, Wieme AD, Boon N, Vandamme P (2021) Genomic aromatic compound degradation potential of novel *Paraburkholderia* species: *Paraburkholderia domus* sp. nov., *Paraburkholderia haematera* sp. Nov. and *Paraburkholderia nemoris* sp. nov. *Prep Biochem Biotechnol* 22:7003. <https://doi.org/10.3390/ijms22137003>
- Wagner AO, Markt R, Puempel T, Illmer P, Insam H, Ebner C (2017) Sample preparation, preservation, and storage for volatile fatty acid quantification in biogas plants. *Eng Life Sci* 17:132–139. <https://doi.org/10.1002/elsc.201600095>
- Wickham H (2016) *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York
- Winkler J, Kao KC (2011) Transcriptional analysis of *Lactobacillus brevis* to n-butanol and ferulic acid stress responses. *PLoS ONE* 6(8):e21438. <https://doi.org/10.1371/journal.pone.0021438>
- Wohl J, Petersen M (2020) Phenolic metabolism in the hornwort *Anthoceros agrestis*: 4-coumarate CoA ligase and 4-hydroxybenzoate CoA ligase. *Plant Cell Rep* 39:1129–1141. <https://doi.org/10.1007/s00299-020-02552-w>
- Xu Y, Chen B, Chao H, Zhou NY (2013) mhpT encodes an active transporter involved in 3-(3-hydroxyphenyl)propionate catabolism by *Escherichia coli* K-12. *Appl Environ Microbiol* 79:6362–6368. <https://doi.org/10.1128/AEM.02110-13>
- Yoo HG, Kwon SY, Karki S, Kwon HJ (2011) A new route to dTDP-6-deoxy-L-talose and dTDP-L-rhamnose: dTDP-L-rhamnose 4-epimerase in *Burkholderia thailandensis*. *Bioorganic Med Chem Lett* 21:3914–3917. <https://doi.org/10.1016/j.bmcl.2011.05.030>
- Yoshida S, Hiraga K, Takehana T, Taniguchi I, Yamaji H, Maeda Y, Toyohara K, Miyamoto K, Kimura Y, Oda K (2016) A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science* 351:1196–1199. <https://doi.org/10.1126/science.aad6359>
- Yu G, Wang LG, Han Y, He QY (2012) clusterProfiler: an R package for comparing biological themes among gene clusters. *Omic J Integr Biol* 16:28–287. <https://doi.org/10.1089/omi.2011.0118>
- Zhang Y, Ezeji TC (2013) Transcriptional analysis of *Clostridium beijerinckii* NCIMB 8052 to elucidate role of furfural stress during acetone butanol ethanol fermentation. *Biotechnol Biofuels* 6:66. <https://doi.org/10.1186/1754-6834-6-66>
- Zhou Y, Lin L, Wang H, Zhang Z, Zhou J, Jiao N (2020) Development of a CRISPR/Cas9-based tool for metabolic engineering of *Pseudomonas putida* for ferulic acid-to-polyhydroxyalkanoate bioconversion. *Commun Biol* 3:1–13. <https://doi.org/10.1038/s42003-020-0824-5>
- Zhu D, Xu L, Sethupathy S, Si H, Ahmad F, Zhang R, Zhang W, Yang B, Sun J (2021) Decoding lignin valorization pathways in the extremophilic *Bacillus ligniniphilus* L1 for vanillin biosynthesis. *Green Chem* 23:9554–9570. <https://doi.org/10.1039/D1GC02692E>

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