Ludwikowski *et al. Annals of Microbiology* (2023) 73:11
https://doi.org/10.1186/s13213-023-01714-x

Annals of Microbiology

UNIVERSITÀ DEGLI STUDI DI MILANO

ORIGINAL ARTICLE

Open Access

Bioconversion of ferulic acid and vanillin to vanillic acid by cold-adapted *Paraburkholderia aromaticivorans* AR20-38: impact of culture conditions

Thomas M. Ludwikowski, Andreas O. Wagner and Rosa Margesin*

Abstract

Purpose Biovalorization of lignin-derived aromatic monomers such as ferulic acid (FA) has attracted considerable interest. The cold-adapted strain *Paraburkholderia aromaticivorans* AR20-38 converts FA to the value-added product vanillic acid (VA), without further VA degradation. The efficiency of the bioconversion of FA to VA was optimized by studying culture conditions.

Methods Various cultivation parameters (agitation, temperature, FA concentration, nutrient supplementation) were assessed to increase biomass production and shorten the cultivation time, while obtaining high VA production yields. The fate of the intermediate vanillin was also studied. Lignin monomers and degradation products (FA, vanillin, VA) were quantified via UV/Vis-HPLC.

Result Full bioconversion of 5 mM FA occurred over a broad temperature range of 5–30 °C. Concentrations up 30 mM FA were utilized as the sole carbon source at 20 °C. Molar VA yields (> 90%) produced from 5 to 12.5 mM FA and from 15 to 17.5 mM FA (82–87%) were not significantly different at 10 °C and 20 °C. The supplementation of the mineral medium with monosaccharides (glucose, fructose, mannose) and/or N-rich complex compounds (yeast extract, casamino acids) resulted in high biomass production, accelerated FA bioconversion, and high molar yields (96–100%). The presence of the N-rich compounds alone or in combination with glucose reduced the incubation time necessary to convert FA to VA. Vanillin, formed as an intermediate during FA degradation, was consumed and converted to VA before FA metabolization, when added in combination with FA. Vanillin bioconversion was significantly accelerated in the presence of glucose.

Conclusion The variation of culture conditions improved the efficiency of the studied strain to convert FA via vanillin to VA and demonstrated remarkable FA bioconversion under varying environmental conditions, especially temperature, substrate concentration, and nutrient availability, which is of importance for potential future application.

Keywords Lignin-derived aromatic monomers, Ferulic acid, Vanillin, Vanillic acid, Bioconversion, Cold-adapted *Paraburkholderia*

*Correspondence:
Rosa Margesin
rosa.margesin@uibk.ac.at
Department of Microbiology, University of Innsbruck, Technikerstrasse 25, 6020 Innsbruck, Austria

Background

Lignin, one of the most abundant polymers on earth (Ganewatta et al. 2019), is the largest renewable reservoir of organic material and could be used for the substitution of petroleum-based fuels and aromatic chemicals

© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

(Chauhan 2020; Wang et al. 2019; Xu et al. 2019). The valorization of lignin is a great challenge due to its complex and heterogeneous structure. Several chemical and physical methods, such as pyrolysis, hydrogenation, selective oxidation, and treatment under hydrothermal and supercritical conditions, have been developed for lignin depolymerization. However, these technologies use expensive chemical catalysts and require extensive separation and purification procedures (Chauhan 2020; Xu et al. 2019 and refs. therein). The biological treatment of the recalcitrant polymer lignin is an ecologically friendly alternative for lignin valorization (Chauhan 2020; Sharma et al. 2018; Xu et al. 2019). Microbial biotransformations are important tools for the renewal of natural resources by conversion into commercially valuable products (Brunati et al. 2004; Mishra et al. 2016). In nature, lignin depolymerization is initiated by fungi, especially wood-rotting basidiomycetes, the produced products (lignin monomers, dimers, and other low molecular mass aromatic compounds) can be utilized efficiently by bacteria (Lubbers et al. 2019; Xu et al. 2019). Cellular constituents, such as bacterial enzymes, are a promising source for complete lignin depolymerization (Chauhan 2020).

Since lignin is a complex matrix of aromatic (phenolic) and aliphatic compounds, it is a rich resource of renewable aromatic compounds that could be of biotechnological interest (Brink et al. 2019; Lubbers et al. 2019). In the context of the bio-valorization of lignin-derived aromatic compounds, ferulic acid (FA) has attracted considerable interest. FA is a very abundant and (almost) ubiquitous plant constituent that can be found in the cell wall of a wide range of vegetation (Rosazza et al. 1995) and thus in common agricultural residues, such as cereal brans and sugar-beet pulp (Oddou et al. 1999). FA bioconversion represents a promising way for the production of commercially valuable fine chemicals such as vanillin and vanillic acid (VA) (Kaur and Chakraborty 2013; Rosazza et al. 1995; Xu et al. 2019). Both compounds are intermediates of microbial FA degradation (Brink et al. 2019; Bugg et al. 2011; Lubbers et al. 2019; Xu et al. 2019). Vanillin is one of the most important components of natural flavors and widely used in the food, pharmaceutical, and medical industry (Kaur and Chakraborty 2013; Rosazza et al. 1995; Xu et al. 2019). VA has a wide range of applications and is used as a flavoring agent and food preservative, in the synthesis of polyester and oxygenated aromatic chemicals, such as vanillin, and in biomedical treatments due to its analeptic and antibacterial activity (Baniahmad et al. 2020; Rosazza et al. 1995; Upadhyay et al. 2020).

For the biotechnological application of FA bioconversion to vanillin and VA by robust cell factories, the stable production of these compounds, without their further degradation to metabolites that enter the TCA cycle, is requested. Their further metabolization can be prevented by genetic engineering (dos Santos et al. 2022; Luziatelli et al. 2019). However, the accumulation of VA from FA degradation by native bacterial (and fungal) strains has been described in a number of studies (e.g., Ashengroph et al. 2012; Brunati et al. 2004; Ghosh et al. 2007; Lu et al. 2020; Mishra et al. 2016). In an earlier study, we described the stable accumulation of high amounts of VA by the cold-adapted bacterial strain *Paraburkholderia aromaticivorans* AR20-38 isolated from Alpine forest soil (Margesin et al. 2021). The potential of representatives of the genus to degrade aromatic compounds has been reported recently (Lee et al. 2019; Vanwijnsberghe et al. 2021). To benefit from bioconversion, high yields from high substrate amounts, in particular under varying environmental conditions (such as fluctuating temperature), are desirable for biotechnological applications. Therefore, it was the aim of this study to optimize the bioconversion of FA to VA by strain *P. aromaticivorans* AR20-38. We assessed various cultivation parameters (agitation, temperature, FA concentration, nutrient supplementation) to increase biomass production and shorten the cultivation time, while obtaining high VA production yields. We also studied the fate of the intermediate vanillin, which could not yet be demonstrated by cultivation.

Materials and methods

Strain

The bacterial strain *P. aromaticivorans* AR20-38 used in this study was isolated from soil from an Alpine coniferous forest site (Franca et al. 2016) and identified as described (Berger et al. 2021; GenBank accession no. MT281269). The whole genome was sequenced and the resulting draft genome sequence of the strain has been described (Poyntner et al. 2020; GenBank BioProject number PRJNA624061). The strain was deposited in the China General Microbiological Culture Collection Center (CGMCC 1.18749). The strain was stored at − 80 °C using ROTI©Store cryovials.

Chemicals

Trans-ferulic acid (FA; Sigma-Aldrich 128,708), vanillin (Merck 818,718), and vanillic acid (VA; Merck 841,025) were of chromatographic pure grade. Stock solutions (0.5–1 M) were prepared in DMSO and stored at 4 °C. Preliminary studies showed that the final amount of DMSO in the cultivation flasks did not affect bacterial growth.

Effect of cultivation parameters (temperature, agitation, and FA concentration) on bioconversion of FA to VA

Biodegradation assays were carried out as previously described (Margesin et al. 2021) in 100-mL Erlenmeyer flasks with screw caps containing 20 mL of mineral salts medium (MM) supplemented with FA as the sole carbon source. To ensure sufficient aeration, the culture flasks were opened regularly under sterile conditions. The pH-neutral MM contained (compositions indicated per liter) $Na_2HPO_4 \times 2H_2O$ (3.5 g), KH_2PO_4 (2 g), $(NH_4)_2SO_4$ (1 g), $MgSO_4 \times 7\ H_2O$ (0.2 g), $Ca(NO_3)_2 \times 4\ H_2O$ (0.05 g), ammonium iron(III) citrate (10 mg), a trace element and a vitamin solution (Margesin and Schinner 1997; Margesin et al. 2021).

For inoculation, a preculture of *P. aromaticivorans* AR20-38 grown in MM containing glucose (2 g/L) as a carbon source was prepared. The bacterial cells were separated by centrifugation (10,000 × g for 10 min), washed twice with sterile MM and suspended in MM. The initial (t0) optical density at 600 nm (OD600) in the inoculated flasks was adjusted to 0.05 by inoculation with an adequate aliquot of the pre-culture. Two negative controls contained (1) sterile medium supplemented with FA and (2) inoculated medium without FA. Growth (OD600), pH of the cultures, and the concentrations of FA and VA were monitored in samples collected at regular time intervals. All experiments were performed in triplicate.

To evaluate the effect of agitation on bioconversion of 5 mM FA, cultivation was done at 20 °C without shaking as well as on a rotary shaker at 150, 200, and 250 rpm. The effect of temperature on bioconversion of 5 mM FA to VA was studied at 150 rpm at temperatures ranging from 1 to 30 °C (5 °C intervals). To determine the effect of FA concentration on FA bioconversions, the strain was cultivated at 10 °C and 20 °C at 150 rpm in MM supplemented with 5, 10, 12.5, 15, 17.5, 20, 25, and 30 mM FA as sole carbon source. The pH of the medium was adjusted to 7.0 after the addition of the various FA concentrations.

Effect of medium composition (nutrient supplements) on bioconversion of FA to VA

The ability of strain *P. aromaticivorans* AR20-38 to utilize various sugars (monosaccharides: L-arabinose, D-fructose, galactose, D-glucose, D-mannose, D-xylose; disaccharides: cellobiose, lactose, maltose, D-sucrose, starch; sugar alcohol: glycerol) and N-rich complex compounds (yeast extract (YE), casamino acids (CAS); thereafter named as N sources) for growth was tested by cultivation in MM supplemented with these compounds (0.2% w/v) at 20 °C and 150 rpm. Next, the strain was grown with various concentrations (0.05%, 0.1%, 0.2%, and 0.3% (w/v)) of D-glucose, D-fructose, D-mannose, YE, and CAS.

To test the effect of various nutrient supplements on bioconversion of 5 mM FA, the strain was grown at 20 °C and 150 rpm in MM containing 5 mM FA and 0.3% (w/v) of D-glucose, D-fructose, D-mannose, YE, or CAS. Each of the monosaccharides and the N sources was tested alone or in combination (Table 1). The control contained 5 mM FA without nutrient supplementation. Sterile controls were prepared additionally. The preculture was obtained as described above. Growth (OD600), pH of the cultures, and the concentrations of FA and VA were monitored in samples collected at regular time intervals.

Effect of vanillin on bacterial growth

The growth-inhibiting effect of vanillin was studied in triplicate at 10 °C and 20 °C at 150 rpm in MM containing

Table 1 Effect of supplementation of MM with nutrients (0.3% (w/v)), alone or in combination, on growth and VA production from 5 mM FA by *P. aromaticivorans* AR20-38 at 20 °C

Nutrient supplement	Growth (OD600)	Molar VA yield (%) from 5 mM FA
Without (control)	0.23 a	98.5 a
Glucose	2.75 c	101.9 a
Fructose	2.90 c	101.4 a
Mannose	2.76 c	100.8 a
Yeast extract	1.59 b	98.9 a
Casamino acids	1.30 b	96.2 a
Glucose + yeast extract	3.92 d	101.9 a
Glucose + casamino acids	4.39 e	95.0 a
Fructose + yeast extract	4.34 e	100.6 a
Fructose + casamino acids	4.44 e	98.1 a
Mannose + yeast extract	3.89 d	100.3 a
Mannose + casamino acids	3.90 d	96.4 a
Glucose	2.75 a	101.9 a
Glucose + yeast extract	3.92 b	101.9 a
Glucose + casamino acids	4.39 c	95.0 a
Fructose	2.90 a	101.4 a
Fructose + yeast extract	4.34 b	100.6 a
Fructose + casamino acids	4.44 b	98.1 a
Mannose	2.76 a	100.8 a
Mannose + yeast extract	3.89 b	100.3 a
Mannose + casamino acids	3.90 b	96.4 a
Yeast extract	1.59 a	98.9 a
Glucose + yeast extract	3.92 b	101.9 ab
Fructose + yeast extract	4.34 c	100.6 ab
Mannose + yeast extract	3.89 b	100.3 ab
Casamino acids	1.30 a	96.2 a
Glucose + casamino acids	4.39 c	95.0 a
Fructose + casamino acids	4.44 c	98.1 a
Mannose + casamino acids	3.90 b	96.4 a

Data represent mean values of three replicates. Different letters (a-f) indicate statistically significant differences ($p \leq 0.05$) between nutrients

0.2% (w/v) glucose and vanillin at concentrations of 0 (= control), 1, 2, 3, 4, and 5 mM.

Effect of medium composition (nutrient supplements) on bioconversion of vanillin to VA

To study vanillin bioconversion, *P. aromaticivorans* AR20-38 was grown in triplicate at 10 °C and 20 °C and 150 rpm in MM containing 5 mM vanillin and supplemented with/without 5 mM FA. Additionally, medium variations were prepared by adding glucose or YE (0.3% w/v). The pH of the medium was adjusted to 7.0 after the addition of the various supplements. The preculture was obtained as described above. Growth (OD600), pH of the cultures, and the concentrations of vanillin, FA, and VA were monitored in samples collected at regular time intervals.

HPLC analysis

The samples for lignin monomer analysis were prepared as described previously (Margesin et al. 2022, 2021) and quantified via UV/Vis-HPLC (220 nm) using a RFQ Fast Acid column (50 × 7.8 mm, Phenomenex, Germany) as shown in Wagner et al. (Wagner et al. 2017). As external standards, FA, VA, and vanillin were used in concentrations of 1, 5, and 10 mM.

Statistical data analysis

The statistical calculations were done using the software Statistica 13. The normal distribution of data was confirmed by the Kolmogorov–Smirnov test. Data were analyzed by ANOVA ($p \leq 0.05$), followed by the post hoc Fisher LSD test ($p \leq 0.05$). Differences between cultivation conditions, growth (OD600), and VA production (molar yield) were considered significant when p was ≤ 0.05.

Results

In all biodegradation experiments, no abiotic losses could be detected over the whole incubation periods (data not shown). Thus, the differences between the initial and the residual FA, vanillin, and VA concentrations had to be attributed to bioconversion by strain *P. aromaticivorans* AR20-38. The initial pH of 7.0 in the culture media was not affected during growth and biodegradation.

Molar VA yields were calculated from the initial concentrations of FA and vanillin and the produced VA concentration. Bioconversion per cell dry mass could not be calculated because cells of strain *P. aromaticivorans* AR20-38 do not sediment sufficiently after centrifugation. However, biomass-related VA production was highest, when OD600 values were low (indicated in tables and figures) and thus followed tendentially the opposite trend of biomass production.

Effect of agitation on FA bioconversion

Shaking of the culture flasks had a significantly positive effect on growth, FA biodegradation and VA production by strain *P. aromaticivorans* AR20-38 compared to the performance under unshaken conditions (Fig. 1). There were no significant differences ($p \leq 0.05$) between the different shaking speeds tested (150, 200, 250 rpm) (Table 2). The highest FA bioconversion was noted after 4 days at 20 °C with shaking, regardless of the shaking speed, while 7 days were needed for the same performance without shaking. However, the shaking speed had no significant effect ($p \leq 0.05$) on the molar VA yield (97–98%) obtained in the stationary growth phase of the strain (Table 2).

Effect of temperature on FA bioconversion

During an incubation period of 18 days, strain *P. aromaticivorans* AR20-38 was able to degrade 5 mM FA and fully convert it into VA over a temperature range of 5–30 °C (Fig. 2). Almost no growth and biodegradation could be observed at 1 °C. An increase in biomass production was accompanied by FA degradation and stable VA accumulation due to FA bioconversion. The intermediate vanillin was not detected at any of the incubation temperatures tested and therefore not excreted from cells.

Biodegradation (bioconversion) was fastest at 20–30 °C, where at least 95% degradation was noted within 3 days, closely followed by 15 °C (4 days), while 7 days were needed at 10 °C. At 5 °C, growth, and thus also biodegradation, were considerably delayed; nonetheless, more than 90% of 5 mM FA were degraded after 16 days. When maximum biomass formation had occurred, there was no significant difference ($p \leq 0.05$) between the molar (98–100%) VA yield produced from FA at temperatures ranging from 5 to 30 °C (Table 3). The highest biomass formation was noted at 5 °C, followed by that produced at 25–30 °C (not significantly different, $p \leq 0.05$) and at 10–20 °C (not significantly different, $p \leq 0.05$). This was also observed in MM containing glucose as the sole carbon source (data not shown).

Effect of FA concentration on bioconversion

Remarkably, strain *P. aromaticivorans* AR20-38 was able to grow well in the presence of 5–30 mM FA at 20 °C (within 20 days); growth was always paralleled by full FA degradation and stable VA production (Fig. 3). Generally, increased FA concentrations resulted in increased biomass formation but delayed FA bioconversion. Full degradation of 5–17.5 mM FA was obtained within 3 days (5 mM), 5 days (10 mM),7 days (12.5 mM), 12 days (15 mM), or 14 days (17.5 mM). Of 20–25 mM and 30 mM FA, less than 5% of the initial concentration was left after 15–16 and 20 days of incubation, respectively. While molar VA yields > 90%

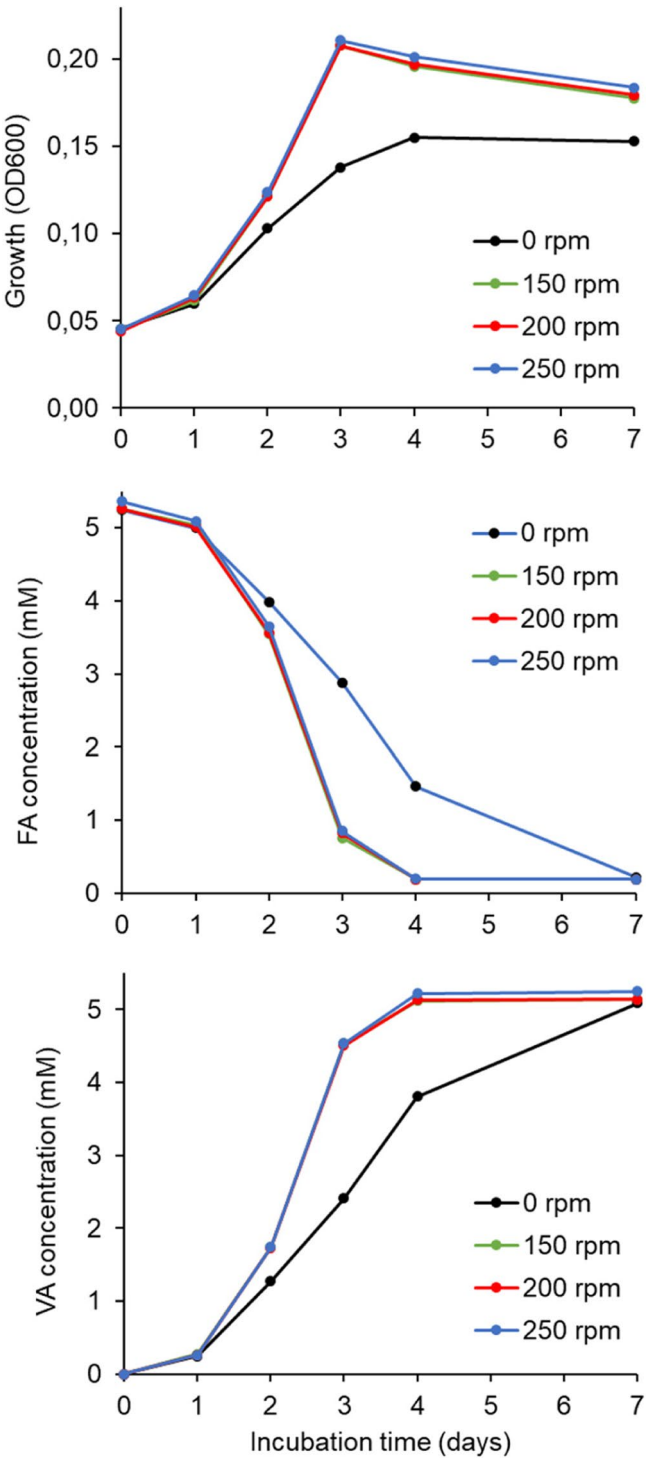

Fig. 1 Effect of agitation on growth (upper panel), degradation of 5 mM FA (middle panel), and VA production (lower pannel) by *P. aromaticivorans* AR20-38. Data represent mean values of three replicates (SDs < 10%)

(91–100%) were obtained from bioconversion of initial FA concentrations of 5–12.5 mM and 15–17.5 mM FA, respectively, only 62% VA (the significantly ($p \leq 0.05$) lowest molar yield compared to that from lower FA concentrations) were produced from 30 mM FA (Fig. 3, Table 4).

Table 2 Effect of aeration on growth and VA production from 5 mM FA by *P. aromaticivorans* AR20-38 at 20 °C

rpm	Growth (OD600)	Molar VA yield (%) from 5 mM FA
0	0.155 a	97.1 a
150	0.208 b	97.7 a
200	0.208 b	97.7 a
250	0.211 b	97.9 a

Data represent mean values of three replicates. Different letters (a, b) indicate statistically significant differences (LSD, $p \leq 0.05$) between shaking speeds

At 10 °C, however, growth and biodegradation were significantly delayed (by 2–3 days) in comparison with the performance at 20 °C: for example, 15 and 28 days were needed for full degradation of 10 and 17.5 mM FA, respectively. Of 20 mM FA, 21% remained untouched after 32 days; however, the stationary growth phase was not yet reached. Molar VA yields > 90% (92–99%) and > 80% (82–87%), respectively, were obtained from bioconversion of initial FA concentrations of 5–12.5 mM and 15–17.5 mM FA, respectively; however, a significantly ($p \leq 0.05$) lower yield of 68% was produced from 20 mM FA (Fig. 3, Table 4). Due to the delayed growth with 20 mM FA, higher concentrations (25 mM and 30 mM) were not tested at 10 °C.

Nonetheless, molar VA yields produced from 5 to 12.5 mM FA (> 90%) and from 15 to 17.5 mM FA (82–87%) were comparable at 10 °C and 20 °C. In fact, there were no significant ($p \leq 0.05$) differences between molar VA yields produced from 5 to 17.5 mM at 10 °C and 20 °C; only yields from 20 mM FA were significantly higher at 20 °C (78%) than at 10 °C (68%) (Fig. 4).

Effect of medium composition (nutrient supplements) on bioconversion of FA to VA

Strain *P. aromaticivorans* AR20-38 was able to utilize all monosaccharides tested (growth was lowest with L-arabinose and D-xylose), glycerol as well as YE and CAS for growth. The tested disaccharides resulted in absent or very weak growth. The highest biomass formation was obtained with the monosaccharides D-fructose, D-glucose, and D-mannose, and with the N sources CAS and YE extract (data not shown). Therefore, these compounds were used to test the effect of nutrient supplementation on FA bioconversion.

Next, the strain was grown with various concentrations (0.05%, 0.1%, 0.2%, and 0.3% (w/v)) of D-glucose, D-fructose, D-mannose, YE, and CAS. *P. aromaticivorans* AR20-38 could utilize all tested compounds in all added concentrations; the higher the concentration, the higher biomass formation was (Fig. 5). Biomass formation from

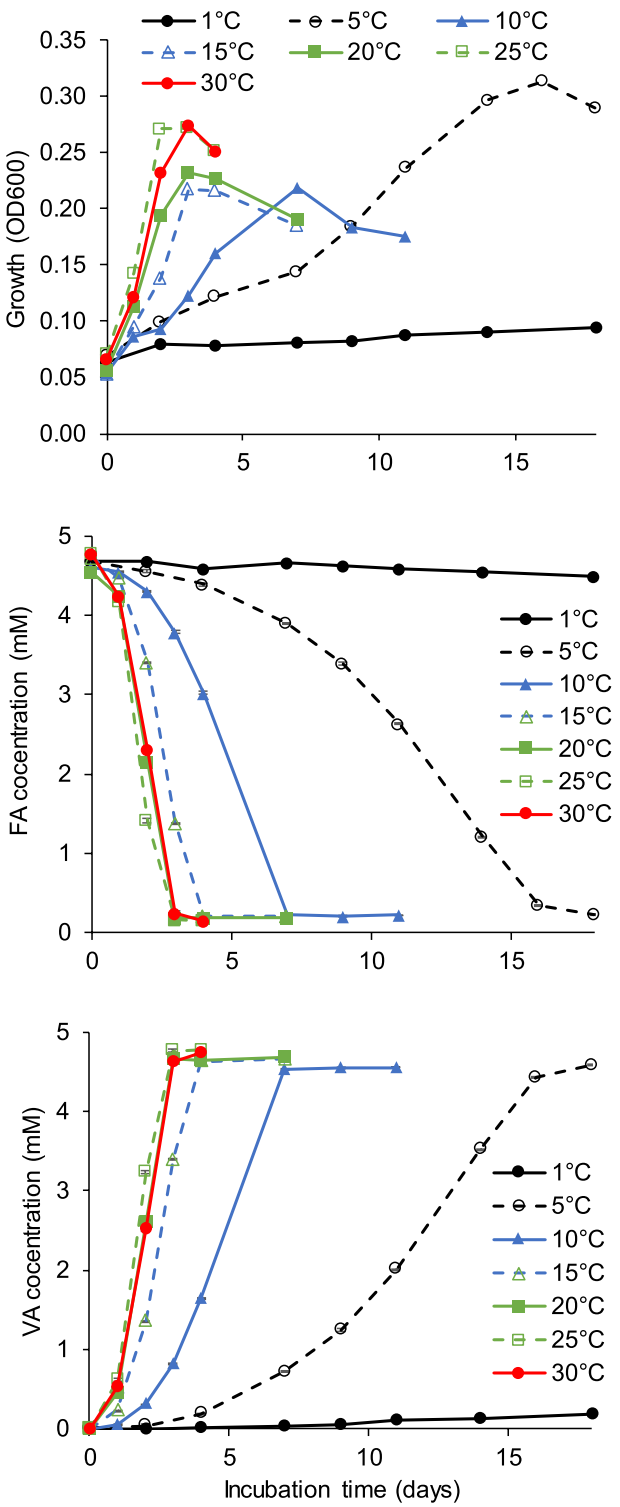

Fig. 2 Effect of temperature on growth (upper panel), degradation of 5 mM FA (middle panel), and VA production (lower pannel) by *P. aromaticivorans* AR20-38. Data represent mean values of three replicates (SDs < 10%)

the three sugars was always comparable and significantly higher than that produced from CAS and YE.

Finally, it was evaluated whether the supplementation of MM with the C or/and N sources, alone or in combination, would stimulate or accelerate 5 mM FA biodegradation at 20 °C. Indeed, the MM supplementation with nutrients influenced growth (incubation time and biomass production) and FA bioconversion significantly (Table 1, Fig. 6). There was no FA release or VA production from any of the tested nutrient supplements as observed in control samples. Biomass production in the stationary growth phases with 5 mM FA as the sole carbon source was almost sixfold higher in the presence of an additional N source, about 12-fold higher in the presence of an additional carbon source (without significant differences between glucose, fructose, and mannose) and even 17–19-fold higher if monosaccharides and N sources were combined. Among additionally added C sources (sugars), the lag phase was shortest in the presence of glucose. The stationary growth phases were obtained after 2 days with glucose and fructose and after 3 days with mannose, which were also needed in case of FA as the sole C source and in case of supplementation with YE and CAS. In case of YE and CAS, growth after 1 day was already close to the stationary phase (Fig. 6).

VA yield produced from 5 mM FA correlated with growth. But in contrast to biomass formation, molar VA yield in the stationary growth phase was not significantly influenced by MM supplementation with nutrients, neither alone nor in combination, and was always in the range of 96–100% (Table 1).

Importantly, nutrient supplements influenced the incubation time required for full FA biodegradation (and thus VA production). With 5 mM FA as the sole carbon source, full FA bioconversion was obtained after 3 days, as it was the case when MM was supplemented with one of the three monosaccharides tested alone or with combinations of mannose with YE or CAS or with fructose and YE. The presence of YE, CAS as well as the combination of glucose with YE or CAS reduced the incubation time from 3 to 2 days. In contrast, the supplementation with fructose and CAS delayed the incubation time to 4 days (Fig. 7).

When 5 mM FA was the sole carbon source, still 65% of the initial concentration was detected after 2 days, while nutrient supplementation had already resulted in more than 90% or even full FA degradation (Fig. 6). After 1 day of incubation, FA degradation was fastest in the presence of YE. Interestingly, the presence of YE or CAS resulted in a comparable bioconversion speed like the presence of monosaccharides despite significantly lower biomass production.

Growth-inhibiting effect of vanillin

The effect of vanillin on the growth of strain *P. aromaticivorans* AR20-38 in MM supplemented with glucose (0.2% w/v) as carbon source was determined at 10 °C and 20 °C, since good growth and FA bioconversion were observed at these two temperatures (Fig. 2).

A decrease in temperature resulted in an increased growth-inhibiting effect of vanillin (Table 5). At 20 °C, 1–4 mM vanillin had no growth-inhibiting effect and did not delay growth; the stationary phase in the presence of 0–4 mM vanillin was reached after 2 days. Only a concentration of 5 mM vanillin resulted in significant ($p \leq 0.05$) growth inhibition of 11% (which was significantly lower than growth inhibition at 10 °C) and an increased lag phase (stationary growth phase after 4 days). At 10 °C, already a concentration of 1 mM vanillin inhibited growth significantly, however only by 8%; 2–5 mM vanillin had a significantly ($p \leq 0.05$) higher negative effect and inhibited growth by 30% on average (Table 5). The higher the vanillin concentration, the longer was the time to reach the stationary growth phase.

Effect of medium composition (nutrient supplements) on bioconversion of vanillin to VA

Figure 8 (A-C) shows the time course of growth as well as VA production from 5 mM vanillin and 5 mM FA at 10 °C and 20 °C. All parameters were delayed at 10 °C compared to 20 °C, however, with the same final result.

Independent of the cultivation temperature, the strain showed almost no growth in the presence of 5 mM vanillin as the sole carbon source or in combination with 5 mM FA, while considerable growth was obtained when the medium was supplemented with 0.3% (w/v) YE. Biomass formation even was approx. threefold (and thus significantly) higher in the presence of 0.3% (w/v) glucose compared to YE. Biomass formation in the presence of FA was higher than in its absence. These results were obtained at both cultivation temperatures. With nutrient supplementation, growth at 10 °C (stationary growth phase after 7–8 days) was delayed compared to growth at 20 °C (stationary growth phase after 3 days of incubation) (Fig. 8A, B).

The medium supplementation with glucose or YE resulted in full vanillin degradation after 2 days at 20 °C and after 7 days at 10 °C, independently of the presence or absence of FA (Fig. 8A, B). The additional presence of 5 mM FA demonstrated clearly that vanillin was always degraded first. FA consumption was completed after 3–4 days at 20 °C and after 8 days at 10 °C. Both, 5 mM vanillin and 5 mM FA, were fully converted to VA, independent of the temperature, with the exception of YE supplementation at 10 °C which resulted in a 90% molar VA yield. Notably, full bioconversion of vanillin, both,

Table 3 Effect of temperature on growth and VA production from 5 mM FA by *P. aromaticivorans* AR20-38

Temperature (°C)	Growth (OD600)	Molar VA yield (%) from 5 mM FA
5	0.314 c	97.7 a
10	0.218 a	99.1 a
15	0.218 a	98.3 a
20	0.232 a	99.5 a
25	0.272 b	99.5 a
30	0.275 b	99.2 a

Data represent mean values of three replicates. Different letters (a-c) indicate statistically significant differences (LSD, $p \leq 0.05$) between temperatures

in the absence and in the presence of FA, was already obtained after 3 days at 20 °C if the medium was supplemented with glucose. In contrast, 8 days were needed with YE supplementation (Table 6).

VA was even produced from 5 mM vanillin in the absence or presence of FA without nutrient supplementation (Fig. 8C). The degradation of 5 mM vanillin as the sole carbon source was accompanied by VA production with molar VA yields of 23% and 31% after 11 days at 10 °C and after 8 days at 20 °C, respectively. In the presence of 5 mM FA, molar VA yields of 15% and 20% were obtained after 11 days at 10 °C and after 8 days at 20 °C, respectively. After 32 days of incubation at 20 °C, the molar VA yield increased to 68 ± 7.3% (Table 6). Full biodegradation could not be obtained, since the incubation time used in this experiment was too short for the strain to reach the stationary growth phase.

No vanillin or FA degradation occurred in sterile controls, nor was there any release of FA, vanillin, or VA from nutrient supplements, which demonstrates that VA production in all experiments had to be attributed to the activity of the studied strain.

Discussion

In a previous study (Margesin et al. 2021), we demonstrated the ability of the cold-adapted strain *P. aromaticivorans* AR20-38 to convert 5 mM and 10 mM FA to high amounts of VA (85–89% molar yield) over a temperature range of 10–30 °C. The proposed pathway, based on the strongly differentially expressed genes in the transcriptome of the strain during bioconversion of FA to VA, has been recently described (Poyntner et al. 2022). FA is metabolized by the activity of 4-coumarate-CoA ligase to feruloyl-CoA. Subsequently, hydroxycinnamoyl-CoA hydratase lyase converts feruloyl CoA to vanillin, and finally, vanillin dehydrogenase converts vanillin to VA.

Here, we report the optimization of the bioconversion of FA and the intermediate product vanillin by increasing biomass production and shortening cultivation time, thereby maintaining high VA production yields. For this, various cultivation parameters (agitation, temperature, FA concentration, nutrient supplementation) were assessed in detail.

The evaluation of the effect of agitation demonstrated clearly the positive effect of shaking. Better oxygen supply under shaking culture conditions resulted in accelerated performance (growth and FA bioconversion) compared to unshaken conditions. However, there was no significant effect between the shaking speeds tested (150–250 rpm); therefore, all further biodegradation experiments were carried out at a shaking frequency of 150 rpm. The cultivation of aerobic microorganisms requires sufficient oxygen supply. Due to its low solubility, dissolved oxygen can become limiting for microbial growth under unfavorable conditions (Schiefelbein et al. 2013). Agitation (shaking) increases the surface area and subsequently the oxygen concentration in the medium.

Temperature is an important factor in biotechnological applications. In this study, we demonstrated the capability of strain *P. aromaticivorans* AR20-38 to degrade and fully convert 5 mM FA into VA over a broad temperature range of 5–30 °C. Despite the significantly highest biomass formation at 5 °C, temperature (5–30 °C, 5 °C intervals) had no significant effect on the molar VA yield (98–100%) obtained from FA bioconversion. Mass transfer rates were dramatically increased with increasing temperatures with no significant difference between incubation temperatures of 20–30 °C. Under those conditions, maximum FA degradation and bioconversion were achieved after 3 days. However, at the time of maximum biomass production (which was delayed at lower temperatures), molar VA yields were not significantly different over the entire temperature range tested. Unfortunately, we cannot compare our data with those obtained by others since the degradation of lignin-derived aromatic monomers has been generally determined under mesophilic or higher temperature conditions, i.e., at temperatures ranging from 28 to 37 °C (Abdelkafi et al. 2006; Ghosh et al. 2007; Lu et al. 2020; Mishra et al. 2016; Ravi et al. 2017; Upadhyay et al. 2020) or even higher (up to 50 °C, Khan et al. 2022). The assessment of the genomic degradation potential of 67 *Paraburkholderia* strains was studied with strains grown at 20 °C (Vanwijnsberghe et al. 2021). There are no data available on low-temperature degradation of lignin-derived compounds by bacteria and yeasts, except for our studies (Berger et al. 2021; Margesin et al. 2022, 2021; Poyntner et al. 2021). As

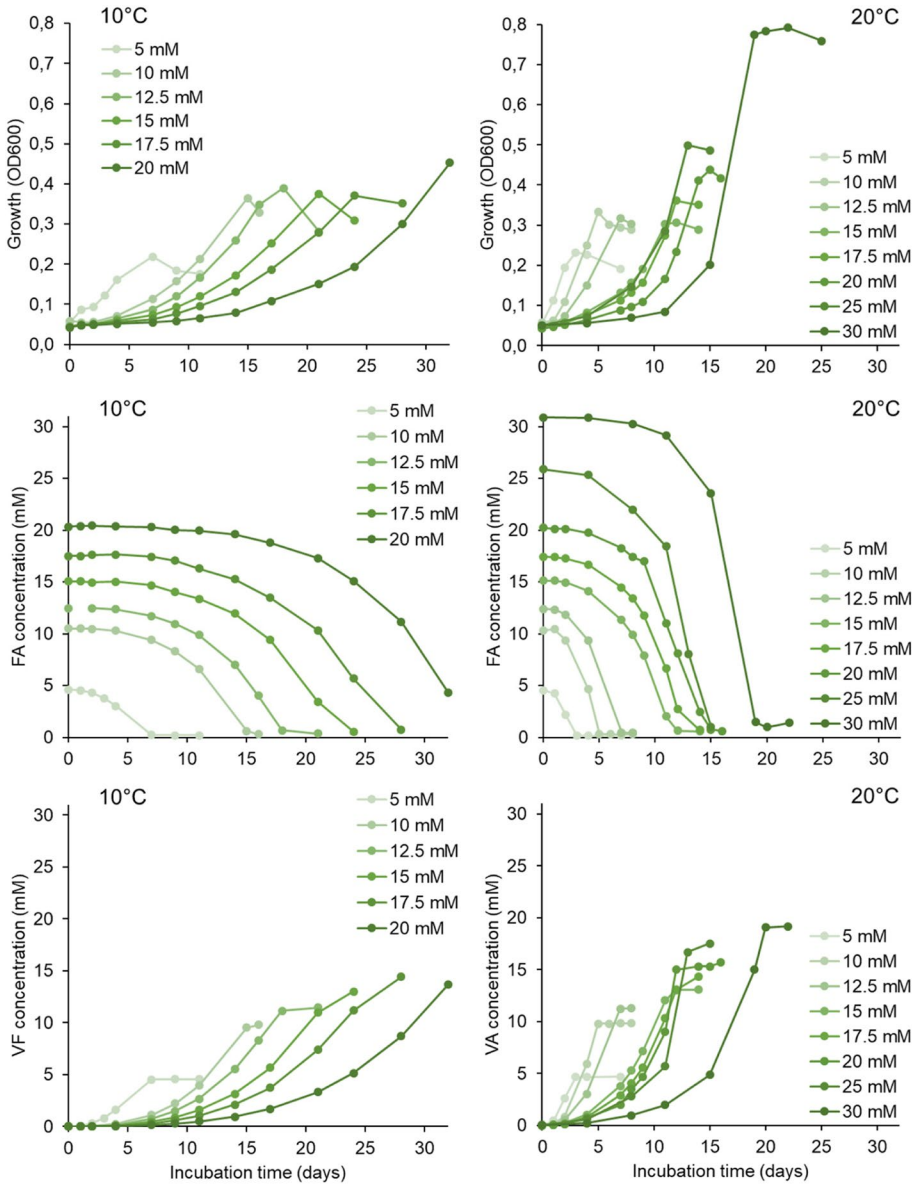

Fig. 3 Effect of FA concentration on growth (upper panels), FA degradation (middle panels), and VA production (lower pannels) at 10 °C (left panels) and 20 °C (right panels) by *P. aromaticivorans* AR20-38. Data represent mean values of three replicates (SDs < 10%)

previously indicated, the ability of the studied strain for low-temperature growth and degradation of lignin-derived aromatic compounds can be attributed to its isolation source, an Alpine coniferous forest site (Franca et al. 2016). The presence of natural aromatic polymers in forest soils, such as lignin, humic substances, and tannins, favors the presence of microbial degraders (Vanwijnsberghe et al. 2021). Knowledge on the microbial contribution to the biodegradation of naturally occurring substances in low-temperature environments is of great ecological importance.

In bioconversion studies, the efficient consumption of high amounts of substrates with a high yield of the desired product is desirable. In our study, we could demonstrate the remarkable tolerance of strain *P. aromaticivorans* AR20-38 towards high concentrations of FA. Remarkably, the VA production yields obtained at 10 °C and 20 °C (82–100%) from FA concentrations up to 17.5 mM were not significantly ($p \leq 0.05$) different. Yields from 20 mM FA were only lower by 10% at 10 °C (68%) than at 20 °C (78%). The yield produced from 30 mM FA at 20 °C was still high (62%). Thus, both FA concentration

Table 4 Effect of FA concentration on growth and VA production from FA by *P. aromaticivorans* AR20-38 at 10 °C and 20 °C

Concentration (mM FA)	Temperature (°C)	Growth (OD600)	Molar VA yield (%)
5.0	10	0.218 a	99.1 d
10.0	10	0.365 b	93.4 c
12.5	10	0.390 b	92.0 c
15.0	10	0.375 b	86.5 b
17.5	10	0.371 b	82.3 b
20.0	10	0.452 c	67.5 a
5.0	20	0.232 a	99.5 g
10.0	20	0.333 b	95.4 f
12.5	20	0.317 b	90.9 ef
15.0	20	0.307 b	86.2 de
17.5	20	0.361 b	82.3 cd
20.0	20	0.438 c	77.5 c
25.0	20	0.499 c	67.7 b
30.0	20	0.792 d	62.0 a

Data represent mean values of three replicates. Different letters (a-f) indicate statistically significant differences (LSD, $p \leq 0.05$) between FA concentrations

and temperature had a significant effect on FA bioconversion. Biomass production increased with increased FA concentrations, which demonstrated the ability of the strain to metabolize high amounts of FA. An increase in FA concentration and a decrease in temperature resulted in a decreased bioconversion yield, which, however, could not be attributed to growth inhibition, since an increase in FA concentrations was accompanied at both temperatures by an increase in biomass production; however, with longer lag phases. Increased lag phase elongation with increasing concentrations of phenolic compounds has also been observed in cultures of *Saccharomyces cerevisiae* (Adeboye et al. 2014). Prolonged lag phases are an indication of stressed cells and might be a defense mechanism that allows bacteria to tolerate stress (Bertrand 2019). Long lag phases could also be an indication of the time required by the strain to repair cell damage and overcome inhibiting effects (Rolfe et al. 2012). In our study, long lag phases before growth on high amounts of FA indicated that the strain adapted successfully to suboptimal conditions. However, once maximum biomass formation was obtained, high VA yields were achieved. Nonetheless, the significantly lower VA yields found with high amounts of FA point to an inhibitory effect on FA metabolization that could not be overcome by time. Further studies on the metabolites produced during FA bioconversion could elucidate the underlying biochemical mechanisms.

To the best of our knowledge, this is the first study to describe the efficient bioconversion of FA to VA from such high FA concentrations. In comparison, Ravi et al. (2017) studied biodegradation and bioconversion of 5 mM FA by *Pseudomonas putida* at 28 °C; the engineered *P. putida* strain degraded 10 mM FA at 30 °C with a molar VA yield of 95% (Upadhyay et al. 2020). Bioconversion of 5 mM FA by *Streptomyces halstedii* at 28 °C (Brunati et al. 2004) and by *Bacillus licheniformis* at 37 °C (Ashengroph et al. 2012) resulted in molar yields of 80% and 60%, respectively. *Halomonas elongata* converted 5 mM FA at 37 °C to VA with a molar yield of 86%; FA concentrations higher than 5 mM inhibited growth by 12%; 30 mM FA reduced growth by 27% (Abdelkafi et al. 2006). The highest molar VA production yield (56%) by *Streptomyces sannanensis* was obtained from 5 mM FA at 28 °C; an increase in temperature (37 °C) and FA concentration (7.5–10 mM) resulted in considerably lower VA yields (Ghosh et al. 2007). The conversion yield of *Paenibacillus lactis* at 37 °C was very low in the presence of 5 mM or 7.5 mM FA compared to 2.5 mM FA (33%) in the presence of FA as the sole carbon source (Mishra et al. 2016). The comparison of these data with those from our study demonstrates impressively that the bioconversion efficiency of *P. aromaticivorans* AR20-38 (tolerance to high amounts of FA and high VA production yields at 10 °C and at 20 °C) is especially remarkable and points to its biotechnological relevance for the production of the value-added product VA from FA.

Media composition is known to affect biodegradation. Since the biomass produced by *P. aromaticivorans* AR20-38 in MM with FA as the sole carbon source was quite low, we assessed the effect of medium supplementation with three C sources (glucose, fructose, mannose) and two N-rich complex compounds (named as N sources; YE, CAS) on growth and FA conversion by the studied strain. MM supplementation with C and N sources alone resulted in an about sixfold and 12-fold higher biomass production, respectively. The combination of C and N sources even enhanced growth by a factor of 17–19. These data demonstrate the significantly positive effect of nutrient supplementation on biomass production. However, this was not the case for FA bioconversion. The molar VA yield was not significantly different in the absence or presence of nutrient supplements and was always in the range of 96–100%. Nonetheless, due to accelerated growth, the incubation time necessary to achieve full bioconversion could be reduced from 3 to 2 days at 20 °C in the presence of YE, CAS, or the combination of glucose with YE or CAS. However, supplementation with YE or CAS might also have provided the strain with additional micronutrients, vitamins, and trace elements.

Medium supplementation with nutrients to increase bacterial biomass formation and FA bioconversion has

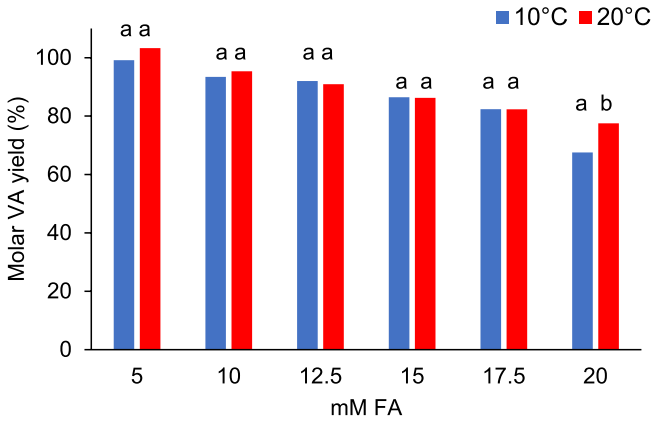

Fig. 4 Effect of temperature (10 °C and 20 °C) on VA production from various FA concentrations (10–20 mM) by *P. aromaticivorans* AR20-38. Data represent mean values of three replicates. Different letters (**a**, **b**) indicate statistically significant differences (LSD, $P \leq 0.05$) between temperatures

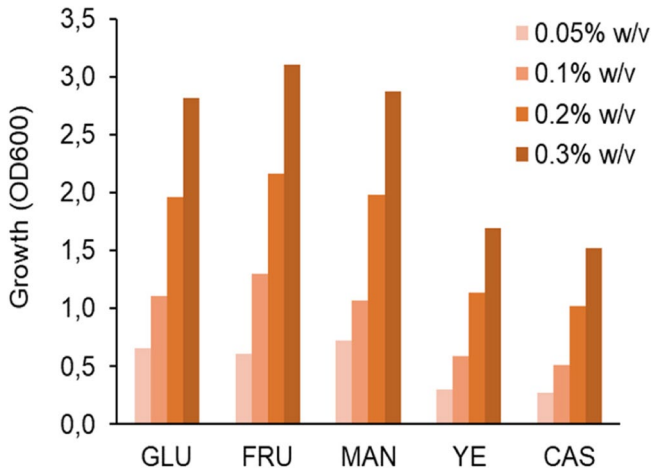

Fig. 5 Effect of various concentrations (0.05–0.3% (w/v)) of monosaccharides (glucose, GLU; fructose, FRU; mannose, MAN), casamino acids (CAS), and yeast extract (YE) on biomass production by *P. aromaticivorans* AR20-38 at 20 °C. Data represent mean values of three replicates (SDs < 10%)

been reported before. Mishra et al. (Mishra et al. 2016) observed that the addition of YE (0.05% w/v) increased the molar yield (57.3 vs. 33%) from 2.5 mM FA and reduced the time required for VA production by *Paenibacillus lactis*, whereas the addition of an additional C source (glucose 0.1% w/v) did not increase VA concentration. Low amounts of YE (0.001% w/v) also increased the yield of VA production and acceleration production time by the transposon mutant *Pseudomonas fluorescens* (Civolani et al. 2000). Glucose supplementation (0.1% w/v) resulted in an increased production of VA by a factor of 2 (however, still only 11.6% molar yield from 10 mM FA) by the fungus *Paecilomyces variotii*, while a decrease in VA accumulation was observed in the presence of the cheaper substrate starch (Ghosh et al. 2006).

During FA biodegradation to VA, bacteria produce the intermediate vanillin (Lu et al. 2020; Lubbers et al. 2019; Ravi et al. 2018; Upadhyay et al. 2020). The analysis of the genome of strain *P. aromaticivorans* AR20-38 demonstrated the presence of two genes encoding for the enzyme involved in the vanillin production from FA (feruloyl-CoA synthase) and the degradation of vanillin to VA was evidenced by the presence of a gene encoding for vanillin dehydrogenase (vanillin I funneling pathway) (eLignin Microbial Database, www.elignindatabase.com; Margesin et al. 2021). However, in all our biodegradation experiments the accumulation of the intermediate vanillin could never be detected. Also, Muheim and Lerch (1999) could not detect vanillin accumulation during FA conversion to VA by *Pseudomonas putida*. This might be attributed to the toxic effect of the phenolic aldehyde vanillin on microorganisms and its rapid degradation or conversion to non- or less toxic compounds like VA (Banerjee

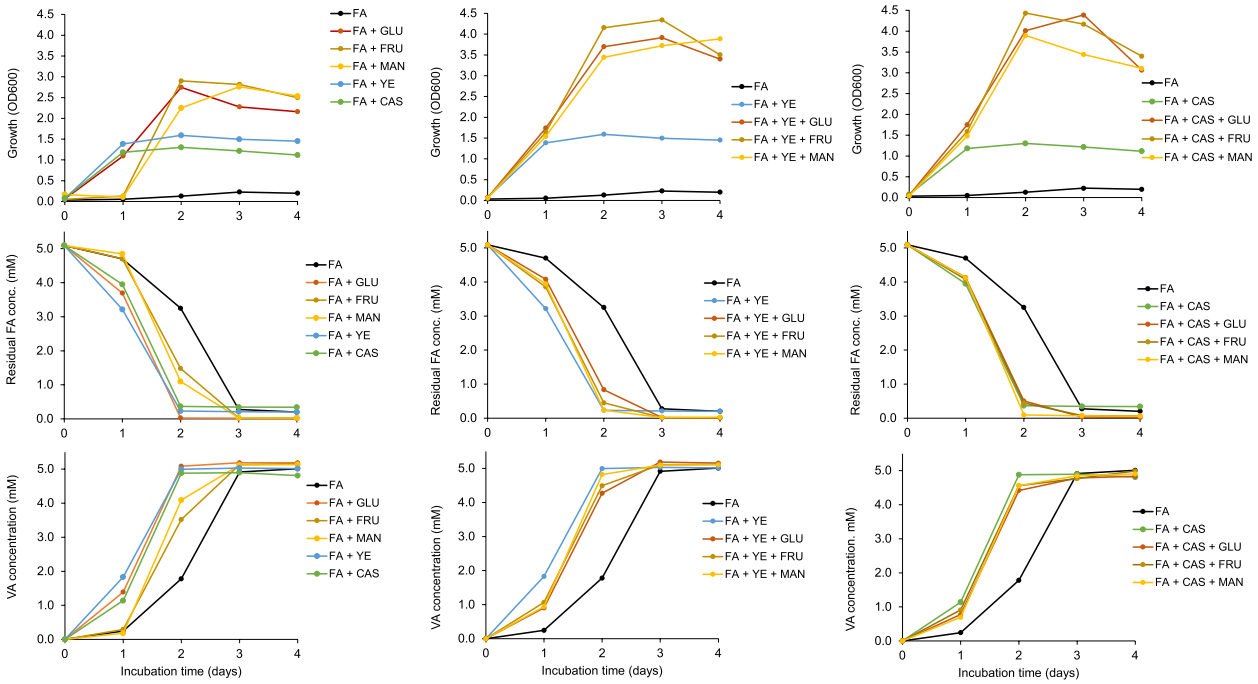

Fig. 6 Effect of supplementation of MM with (0.3% w/v) glucose (GLU), fructose (FRU), mannose (MAN), yeast extract (YE), and casamino acids (CAS) (right panels); YE in combination with monosaccharides (middle panels), or CAS in combination with monosaccharides (right panels) on growth (top panels), 5 mM FA degradation (middle panels), and VA production (bottom panels) by *P. aromaticivorans* AR20-38 at 20 °C. Data represent mean values of three replicates (SDs < 10%)

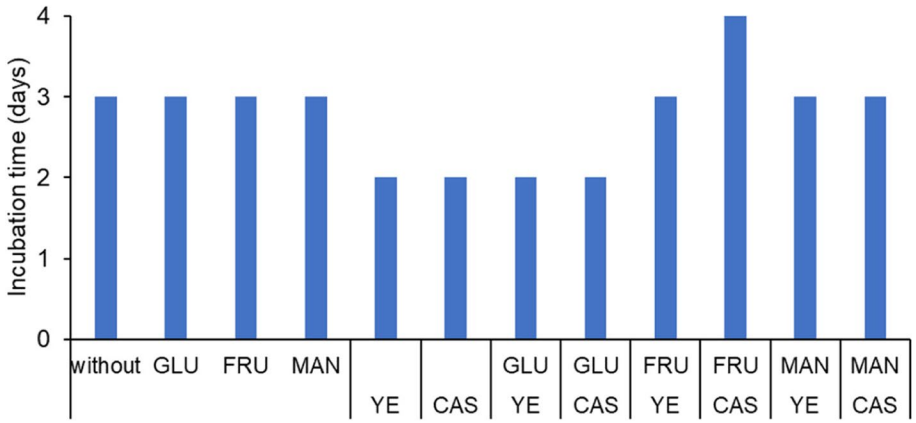

Fig. 7 Effect of incubation time on molar VA yield (%) produced from 5 mM FA by *P. aromaticivorans* AR20-38 at 20 °C in MM supplemented with various nutrients

and Chattopadhyay 2019; Kaur et al. 2013; Lubbers et al. 2019; Luziatelli et al. 2019; Ravi et al. 2017).

Therefore, we studied the effect of vanillin on the growth of strain *P. aromaticivorans* AR20-38 at two temperatures (10 °C and 20 °C). The growth-inhibiting effect of vanillin was significantly influenced by temperature and vanillin concentration. While concentrations of 2–5 mM vanillin inhibited growth at 10 °C by 30% on average, only a concentration of 5 mM vanillin resulted in comparatively slight growth inhibition (11%) at 20 °C. Thus, a decrease in temperature and an increase in vanillin concentration resulted in reduced performance, paralleled by longer lag phases (the same effect was noted with increased FA concentrations, see above). Longer exposure times with the membrane-active compound vanillin (due to longer lag phases) might have resulted in

Table 5 Effect of vanillin concentration (0–5 mM) on relative growth inhibition of *P. aromaticivorans* AR20-38 in MM with glucose (0.2% w/v) at 10 °C (100%: OD600 = 2.74 ± 0.02) and 20 °C (100%: OD600 = 2.03 ± 0.04)

Vanillin conc. (mM)	Temperature (°C)	Relative growth (%)
0	10	100.0 c
1	10	91.8 b
2	10	69.3 a
3	10	69.2 a
4	10	70.1 a
5	10	70.0 a
0	20	100.0 b
1	20	103.3 b
2	20	102.0 b
3	20	98.5 b
4	20	100.0 b
5	20	89.4 a

Data represent mean values of three replicates. Different letters (a-c) indicate statistically significant differences (LSD, $p \leq 0.05$) between vanillin concentrations

negative interactions of vanillin with the cell membrane of the studied strain. The antibacterial activity of vanillin was found to be dependent on the exposure time, concentration, and the target microorganisms (Fitzgerald et al. 2004).

Since the ability of strain *P. aromaticivorans* AR20-38 to produce the intermediate vanillin during FA biodegradation was evident from genomic data (Margesin et al. 2021), we assessed vanillin biodegradation in the absence and presence of FA as well as in the absence and presence of the nutrient supplements glucose and YE. Indeed, VA production from vanillin and from FA occurred at both temperatures tested (10 °C and 20 °C). Full bioconversion of vanillin, independent of the presence of FA, was obtained in the presence of an additional nutrient supplement (glucose or YE). Significantly accelerated vanillin consumption occurred, however, only in the presence of glucose at 20 °C. VA was even produced from 5 mM vanillin in the absence or presence of FA without nutrient supplementation; however, no full bioconversion could be obtained since, in contrast to nutrient-supplemented assays, the stationary growth phase was not reached during the studied incubation period. Our data show clearly (1) that strain *P. aromaticivorans* AR20-38 converted both FA and vanillin to VA, which confirmed our genomic data (Margesin et al. 2021), and (2) that vanillin was always consumed first (i.e., before FA). This demonstrates the bacterial response to the toxic compound vanillin by its quick elimination, as described before (Muheim and Lerch 1999; Ravi et al. 2017). The elimination by degradation and conversion to VA was favored by a short lag phase and high amounts of biomass produced in the presence of nutrients.

Conclusion

The data reported in this study demonstrate a number of remarkable degradation properties of the cold-adapted strain *Paraburkholderia aromaticivorans* AR20-38. Its ability to accumulate VA by FA bioconversion has been described before (Margesin et al. 2021). In this study, the variation of culture conditions led to the following findings and production improvements:

- Agitation favors growth and FA bioconversion; however, the studied strain is not sensitive to the agitation speed (150–250 rpm), which facilitates its handling.
- The strain has an excellent performance to completely convert 5 mM FA over a broad temperature range of 5–30 °C, with the fastest activity at 15–30 °C (3–4 days), followed by 10 °C (7 days) and 5 °C (16 days). This allows application in environments with fluctuating temperature conditions.
- The strain is able to handle very high concentrations (up to 30 mM) of FA. Despite delayed growth and biodegradation at 10 °C compared to 20 °C, there were no significant ($p \leq 0.05$) differences between molar VA yields produced from 5 to 17.5 mM FA at 10 °C and 20 °C. This demonstrates the excellent performance of the strain at low and moderate temperatures.
- The presence of additional nutrient supplements in the MM resulted in accelerated and full bioconversion of 5 mM FA. Interestingly, the strain does not require a specific nutrient, which also facilitates VA production by saving time. Nonetheless, nutrient supplementation did not influence the molar yield, which points to the robustness of the strain.
- Biomass production could be significantly increased with higher FA concentrations and with nutrient supplementation. However, biomass increase did not influence VA yield. This is of advantage when VA production should be obtained (from natural sources) under varying nutrient or environmental conditions.
- Our studies proved the ability of the strain to utilize and convert vanillin, the intermediate produced during bacterial FA degradation, both at 10 °C and 20 °C. Vanillin was always consumed first.

Our study adds knowledge on the utilization of lignin-derived aromatic compounds by the bacterial genus *Paraburkholderia* under varying environmental conditions, especially temperature, substrate concentration, and nutrient availability, which is of importance for

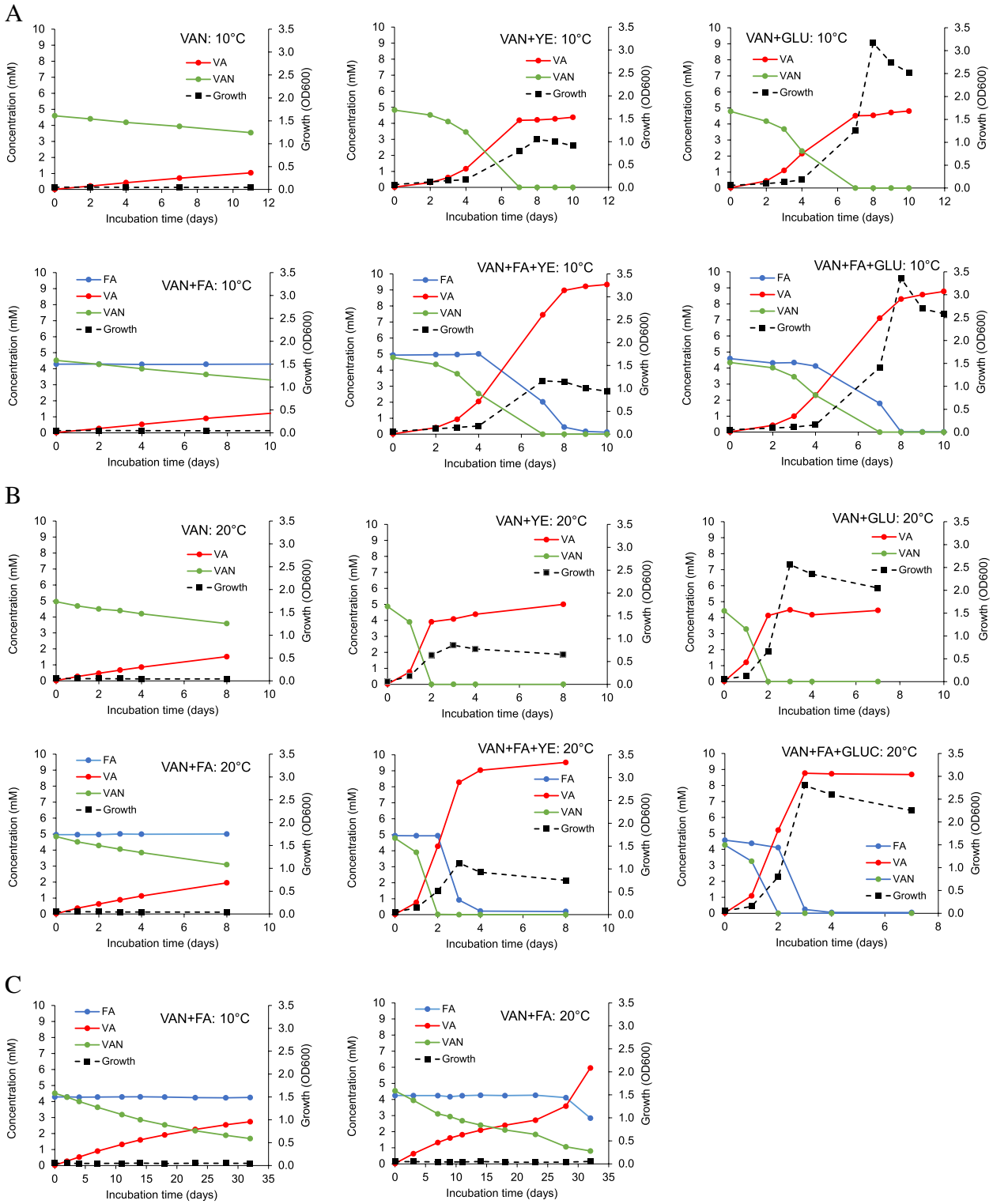

Fig. 8 **A** Effect of glucose (0.3% w/v) and YE (0.3% w/v) on growth and bioconversion of 5 mM vanillin to VA in the absence (top panels) or presence (bottom panels) of 5 mM FA by *P. aromaticivorans* AR20-38 at 10 °C. Data represent mean values of three replicates (SDs < 10%). **B** Effect of glucose (0.3% w/v) and YE (0.3% w/v) on bioconversion of 5 mM vanillin to VA in the absence (top panels) or presence (bottom panels) of 5 mM FA by *P. aromaticivorans* AR20-38 at 20 °C. Data represent mean values of three replicates (SDs < 10%). **C** Bioconversion of 5 mM vanillin to VA after 32 days of incubation in the absence or presence of 5 mM FA (in the absence of nutrient supplements) by *P. aromaticivorans* AR20-38 at 10 °C (left panel) and 20 °C (right panel). Data represent mean values of three replicates (SDs < 10%)

Table 6 VA production from 5 mM vanillin in the absence or presence of 5 mM FA and nutrient supplements by *P. aromaticivorans* AR20-38 at 10 °C and 20 °C. Data represent mean values of three replicates

Vanillin (mM)	FA (mM)	Nutrient supplement	Temperature (°C)	Incubation time (days)	Growth (OD600)	Molar VA yield (%) from	
						5 mM vanillin	5 mM vanillin + 5 mM FA
5	0	Without	10	11	0.047	22.7 a	
5	0	Yeast extract	10	10	1.043	90 b	
5	0	Glucose	10	10	3.167	100.5 c	
5	5	Without	10	11	0.048		15.0 a
				32	0.052		31.1 b
5	5	Yeast extract	10	10	1.163		96.1 c
5	5	Glucose	10	10	3.357		95.6 c
5	0	Without	20	8	0.048	30.6 a	
5	0	Yeast extract	20	8	0.855	101.6 b	
5	0	Glucose	20	3	2.560	101.5 b	
5	5	without	20	8	0.051		19.9 a
				32	0.051		67.7 b
5	5	Yeast extract	20	8	1.130		97.9 c
5	5	Glucose	20	3	2.800		99.1 c

Different letters (a-c) indicate statistically significant differences (LSD, $p \leq 0.05$) between vanillin concentrations

potential future application. Our study adds also knowledge on the capability and potential of cold-adapted microorganisms (Collins and Margesin 2019) for the formation of value-added products from natural resources like lignin. It has to be emphasized that the remarkable biodegradation capacity described in this study was obtained with a native and not with an engineered strain. Further transcriptomic and in vitro studies with resting cells and cell-free enzyme extracts should elucidate the expression and activity of enzymes involved in vanillin and FA bioconversion. FA bioconversion in agricultural residues will also be examined.

Acknowledgements
We thank P. Thurnbichler for skillful technical assistance. The authors thank the publishing fund of the Universität Innsbruck for financial support.

Authors' contributions
R.M. conceptualized the study, designed the degradation experiments, and wrote the manuscript. T.M.L. performed and analyzed the degradation experiments. A.O.W. designed and performed the HPLC analyses and analyzed the data. All authors contributed significantly to the manuscript, read, and agreed to the published version of the manuscript.

Funding
Open-access funding is provided by the University of Innsbruck.

Availability of data and materials
All data generated and analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All of the authors have read and approved the manuscript. This work has not been published previously, nor is it being considered by any other peer-reviewed journal.

Competing interests
The authors declare that they have no competing interests.

Received: 30 November 2022 Accepted: 3 February 2023
Published online: 13 March 2023

References

Abdelkafi S, Sayadi S, Gam ZBA, Casalot L, Labat M (2006) Bioconversion of ferulic acid to vanillic acid by *Halomonas elongata* isolated from table-olive fermentation. FEMS Microbiol Lett 262(1):115–120. https://doi.org/10.1111/j.1574-6968.2006.00381.x.

Adeboye PT, Bettiga M, Olsson L (2014) The chemical nature of phenolic compounds determines their toxicity and induces distinct physiological responses in Saccharomyces cerevisiae in lignocellulose hydrolysates. AMB Express 4:46. https://doi.org/10.1186/s13568-014-0046-7.

Ashengroph M, Nahvi I, Zarkesh-Esfahani H, Momenbeik F (2012) Novel strain of SHL1 with potential converting ferulic acid into vanillic acid. Ann Microbiol 62(2):553–558. https://doi.org/10.1007/s13213-011-0291-9.

Banerjee G, Chattopadhyay P (2019) Vanillin biotechnology: the perspectives and future. J Sci Food Agric 99(2):499–506. https://doi.org/10.1002/jsfa.9303.

Baniahmad B, Safaeian L, Vaseghi G, Rabbani M, Mohammadi B (2020) Cardioprotective effect of vanillic acid against doxorubicin-induced cardiotoxicity in rat. Res Pharm Sci 15(1):87–96. https://doi.org/10.4103/1735-5362.278718.

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(1):87–98. https://doi.org/10.1007/s12223-020-00825-1.

Bertrand RL (2019) Lag phase is a dynamic, organized, adaptive, and evolvable period that prepares bacteria for cell division. J Bacteriol 201(7):e00697-e718. https://doi.org/10.1128/JB.00697-18.

Brink DP, Ravi K, Liden G, Gorwa-Grauslund MF (2019) Mapping the diversity of microbial lignin catabolism: experiences from the eLignin database. Appl Microbiol Biotechnol 103(10):3979–4002. https://doi.org/10.1007/s00253-019-09692-4.

Brunati M, Marinelli F, Bertolini C, Gandolfi R, Daffonchio D, Molinari F (2004) Biotransformations of cinnamic and ferulic acid with actinomycetes. Enzyme Microb Technol 34(1):3–9. https://doi.org/10.1016/j.enzmictec.2003.04.001.

Bugg TDH, Ahmad M, Hardiman EM, Rahmanpour R (2011) Pathways for degradation of lignin in bacteria and fungi. Nat Prod Rep 28(12):1883–1896. https://doi.org/10.1039/c1np00042j.

Chauhan PS (2020) Role of various bacterial enzymes in complete depolymerization of lignin: a review. Biocatal Agric Biotechnol 23 doi:https://doi.org/10.1016/j.bcab.2020.101498.

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(6):2311–2317. https://doi.org/10.1128/aem.66.6.2311-2317.2000.

Collins T, Margesin R (2019) Psychrophilic lifestyles: mechanisms of adaptation and biotechnological tools. Appl Microbiol Biotechnol 103(7):2857–2871. https://doi.org/10.1007/s00253-019-09659-5.

dos Santos OAL, Goncalves TA, Sodre V, Vilela N, Tomazetto G, Squina FM, Garcia W (2022) Recombinant expression, purification and characterization of an active bacterial feruloyl-CoA synthase with potential for application in vanillin production. Protein Expr Purif 197 doi:https://doi.org/10.1016/j.pep.2022.106109.

Fitzgerald DJ, Stratford M, Gasson MJ, Ueckert J, Bos A, Narbad A (2004) Mode of antimicrobial action of vanillin against *Escherichia coli, Lactobacillus plantarum* and *Listeria innocua*. J Appl Microbiol 97(1):104–113. https://doi.org/10.1111/j.1365-2672.2004.02275.x.

Franca L, Sannino C, Turchetti B, Buzzini P, Margesin R (2016) Seasonal and altitudinal changes of culturable bacterial and yeast diversity in Alpine forest soils. Extremophiles 20(6):855–873. https://doi.org/10.1007/s00792-016-0874-2.

Ganewatta MS, Lokupitiya HN, Tang CB (2019) Lignin biopolymers in the age of controlled polymerization. Polymers 11(7) doi:https://doi.org/10.3390/polym11071176.

Ghosh S, Sachan A, Mitra A (2006) Formation of vanillic acid from ferulic acid by *Paecilomyces variotii* MTCC 6581. Curr Sci 90(6):825–829.

Ghosh S, Sachan A, Sen SK, Mitra A (2007) Microbial transformation of ferulic acid to vanillic acid by *Streptomyces sannanensis* MTCC 6637. J Ind Microbiol Biotechnol 34(2):131–138. https://doi.org/10.1007/s10295-006-0177-1.

Kaur B, Chakraborty D (2013) Biotechnological and molecular approaches for vanillin production: a review. Appl Biochem Biotechnol 169(4):1353–1372. https://doi.org/10.1007/s12010-012-0066-1.

Kaur B, Chakraborty D, Kumar B (2013) Phenolic biotransformations during conversion of ferulic acid to vanillin by lactic acid bacteria. Biomed Res Int 2013 doi:https://doi.org/10.1155/2013/590359.

Khan SI, Zarin A, Ahmed S, Hasan F, Belduz AO, Canakci S, Khan S, Badshah M, Farman M, Shah AA (2022) Degradation of lignin by *Bacillus altitudinis* SL7 isolated from pulp and paper mill effluent. Water Sci Technol 85(1):420–432. https://doi.org/10.2166/wst.2021.610.

Lee Y, Lee Y, Jeon CO (2019) Biodegradation of naphthalene, BTEX, and aliphatic hydrocarbons by *Paraburkholderia aromaticivorans* BN5 isolated from petroleum-contaminated soil. Sci Rep 9 doi:https://doi.org/10.1038/s41598-018-36165-x.

Lu P, Wang WN, Zhang GX, Li W, Jiang AJ, Cao MJ, Zhang XY, Xing K, Peng X, Yuan B, Feng ZZ (2020) Isolation and characterization marine bacteria capable of degrading lignin-derived compounds. Plos One 15(10) doi:https://doi.org/10.1371/journal.pone.0240187.

Lubbers RJM, Dilokpimol A, Visser J, Makela MR, Hilden KS, de Vries RP (2019) A comparison between the homocyclic aromatic metabolic pathways from plant-derived compounds by bacteria and fungi. Biotechnol Adv 37(7) doi:https://doi.org/10.1016/j.biotechadv.2019.05.002.

Luziatelli F, Brunetti L, Ficca AG, Ruzzi M (2019) Maximizing the efficiency of vanillin production by biocatalyst enhancement and process optimization. Front Bioeng Biotechnol 7 doi:https://doi.org/10.3389/fbioe.2019.00279.

Margesin R, Ludwikowski TM, Kutzner A, Wagner AO (2022) Low-temperature biodegradation of lignin-derived aromatic model monomers by the cold-adapted yeast *Rhodosporidiobolus colostri* isolated from Alpine forest soil. Microorganisms 10(3) doi:https://doi.org/10.3390/microorganisms10030515.

Margesin R, Schinner F (1997) Bioremediation of diesel-oil-contaminated alpine soils at low temperatures. Appl Microbiol Biotechnol 47(4):462–468. https://doi.org/10.1007/s002530050957.

Margesin R, Volgger G, Wagner AO, Zhang DC, 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(7):2967–2977. https://doi.org/10.1007/s00253-021-11215-z.

Mishra S, Kullu M, Sachan A, Vidyarthi AS, Sachan SG (2016) Bioconversion of ferulic acid to vanillic acid by *Paenibacillus lactis* SAMS-2001. Ann Microbiol 66(2):875–882. https://doi.org/10.1007/s13213-015-1175-1.

Muheim A, Lerch K (1999) Towards a high-yield bioconversion of ferulic acid to vanillin. Appl Microbiol Biotechnol 51(4):456–461. https://doi.org/10.1007/s002530051416.

Oddou J, Stentelaire C, Lesage-Meessen L, Asther M, Ceccaldi BC (1999) Improvement of ferulic acid bioconversion into vanillin by use of high-density cultures of *Pycnoporus cinnabarinus*. Appl Microbiol Biotechnol 53(1):1–6. https://doi.org/10.1007/s002530051605.

Poyntner C, Kutzner A, Margesin R (2021) Biodegradation potential and putative catabolic genes of culturable bacteria from an Alpine deciduous forest site. Microorganisms 9(9) doi:https://doi.org/10.3390/microorganisms9091920.

Poyntner C, Ludwikowski TM, Wagner AO, Margesin R (2022) Transcriptome profiling of *Paraburkholderia aromaticivorans* AR20-38 during ferulic acid bioconversion. AMB Express 12:148 https://doi.org/10.1186/s13568-022-01487-7.

Poyntner C, Zhang DC, 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(27) doi:https://doi.org/10.1128/mra.00463-20.

Ravi K, Garcia-Hidalgo J, Gorwa-Grauslund MF, Liden G (2017) Conversion of lignin model compounds by *Pseudomonas putida* KT2440 and isolates from compost. Appl Microbiol Biotechnol 101(12):5059–5070. https://doi.org/10.1007/s00253-017-8211-y.

Ravi K, Garcia-Hidalgo J, Nobel M, Gorwa-Grauslund MF, Liden G (2018) Biological conversion of aromatic monolignol compounds by a *Pseudomonas* isolate from sediments of the Baltic Sea. AMB Express 8 doi:https://doi.org/10.1186/s13568-018-0563-x.

Rolfe MD, Rice CJ, Lucchini S, Pin C, Thompson A, Cameron ADS, Alston M, Stringer MF, Betts RP, Baranyi J, Peck MW, Hinton JCD (2012) Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. J Bacteriol 194(3):686–701 doi/https://doi.org/10.1128/JB.06112-11.

Rosazza JPN, Huang Z, Dostal L, Volm T, Rousseau B (1995) Review: biocatalytic transformations of ferulic acid: An abundant aromatic natural product. J Ind Microbiol 15(6):457–471. https://doi.org/10.1007/bf01570016.

Schiefelbein S, Frohlich A, John GT, Beutler F, Wittmann C, Becker J (2013) Oxygen supply in disposable shake-flasks: prediction of oxygen transfer rate, oxygen saturation and maximum cell concentration during aerobic growth. Biotechnol Lett 35(8):1223–1230. https://doi.org/10.1007/s10529-013-1203-9.

Sharma A, Kumar R, Rathi M, Bhatia D, Malik DK (2018) Lignocellulose biodegradation: an advance technology for sustainable environment. Biosci Biotechnol Res Commun 11(4):634–637. https://doi.org/10.21786/bbrc/11.4/14.

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(4):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. Int J Mol Sci 22(13) doi: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(2):132–139. https://doi.org/10.1002/elsc.201600095.

Wang HL, Pu YQ, Ragauskas A, Yang B (2019) From lignin to valuable products-strategies, challenges, and prospects. Biores Technol 271:449–461. https://doi.org/10.1016/j.biortech.2018.09.072.
Xu ZX, Lei P, Zhai R, Wen ZQ, Jin MJ (2019) Recent advances in lignin valorization with bacterial cultures: microorganisms, metabolic pathways, and bio-products. Biotechnol Biofuels 12 doi:https://doi.org/10.1186/s13068-019-1376-0.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions