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Engineered Passive Glucose Uptake in *Pseudomonas taiwanensis* VLB120 Increases Resource Efficiency for Bioproduction

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ABSTRACT

Glucose is the most abundant monosaccharide and a principal substrate in biotechnological production processes. In *Pseudomonas*, this sugar is either imported directly into the cytosol or first oxidised to gluconate in the periplasm. While gluconate is taken up via a proton-driven symporter, the import of glucose is mediated by an ABC-type transporter, and hence both require energy. In this study, we heterologously expressed the energy-independent glucose facilitator protein (Glf) from *Zymomonas mobilis* to replace the native energy-demanding glucose transport systems, thereby increasing the metabolic energy efficiency. The implementation of passive glucose uptake in engineered production strains significantly increased product titres and yields of the two different aromatic products, cinnamic acid (+10%–15%) and resveratrol (+26%; 18.1 mg/g) in batch cultures.

1 | Introduction

The uptake of carbohydrates is a fundamental process of microbial life. Especially for the uptake of sugars across biological membranes, a plethora of different sugar utilisation and transport systems have evolved in bacteria (Jeckelmann and Erni 2020) in high dependency on the respective ecological niche. Biotechnological processes usually differ significantly from the microorganism's natural habitats, for example, by high concentrations of carbon sources and a lack of microbial competition. It is thus not surprising that the native sugar uptake and metabolism are not necessarily ideal for the applied bioprocess. In *Pseudomonas*, glucose enters the periplasm from the extracellular space via porins like one of four OprB (Wylie and Worobec 1995). It is subsequently taken up into the cytosol via ATP-binding cassette (ABC) transporter GtsABCD at the expense of ATP (del Castillo et al. 2007; Thomas and Tampé 2020) or oxidised to gluconate by periplasmic glucose dehydrogenase (GDH, Gcd) and gluconolactonases (Nerke et al. 2024). Periplasmic oxidation of glucose allows pseudomonads to shunt electrons via the pyrroloquinoline quinone (PQQ) cofactor directly into the respiratory chain (An and Moe 2016) and is considered the major route for glucose utilisation in many pseudomonads (Kohlstedt and Wittmann 2019). Periplasmic gluconate can further be oxidised to 2-ketogluconate by the gluconate 2-dehydrogenase complex (Gad; PP_3382-3384) in some pseudomonads (Kohlstedt and Wittmann 2019; Volke

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Abbreviations: Gcd, glucose dehydrogenase; Glf, glucose facilitator protein; GRC3, genome-reduced *chassis* strain number 3; MC-III, malonyl-CoA *chassis* strain number 3; MC-IV, malonyl-CoA *chassis* strain number 4; MSM, mineral salt medium.

et al. [2023]. *Pseudomonas taiwanensis* VLB120, a well-established bioproduction platform, lacks this second periplasmic oxidation. In contrast to GtsABCD, the gluconate transporter GntP/GntT or 2-ketogluconate transporters KguT both belong to the major facilitator superfamily (MFS) proton symporters (del Castillo et al. [2007]) and are thus also energy-dependent (Lagarde [1977]).

After translocation into the cytosol, glucose is phosphorylated by glucokinase (Glk) and subsequently converted by one of the three glucose 6-phosphate dehydrogenase (Zwf) isoenzymes (Volke, Olavarría, and Nikel [2021]) and 6-phosphogluconolactonase (Pgl) to yield 6-phosphogluconate, which is an intermediate of the EDEMP cycle (Nikel et al. [2015]) (Figure [1]). Regulation of glucose uptake and sugar catabolism is controlled *inter alia* by the two-component system response regulator GltR-II (PP_1012 in *Pseudomonas putida* KT2440; PVLB_20105 in *P. taiwanensis* VLB120) and the repressor HexR (PP_1021; PVLB_20065) (del Castillo, Duque, and Ramos [2008]; Lim et al. [2022]; Udaondo et al. [2018]). 2-Keto-3-deoxy-6-phosphogluconate (KDPG) is one of the effectors of HexR (Kim, Jeon, and Park [2008]) whose deletion has been shown to be beneficial for the production of *cis,cis*-muconate in strains lacking Gcd due to the derepression of the intracellular catabolic genes (Bentley et al. [2020]; Rorrer et al. [2022]). In certain bioprocesses, excessive periplasmic oxidation of glucose to gluconate via gluconolactone is disadvantageous due to the acidification of the culture broth as this can inhibit microbial growth or require titration of base in pH-controlled cultivations. To avoid massive pH fluctuations or the need of excessive titration of base and acid while simultaneously enabling the application of higher glucose concentration in batch cultivations, the respective *gcd* gene can be deleted and, depending on the metabolic context, this may even lead to improved production, for example, muconic acid, polyhydroxyalkanoates and polyketides to name a few (Bentley et al. [2020]; Poblete-Castro et al. [2013]; Schwanemann et al. [2023]) or avoid the unwanted bypassing conversion of alternative dimeric carbohydrates (Dvořák and de Lorenzo [2018]). However, this deletion may cause detrimental effects because gluconate formation is involved in a wide regulatory network (Volke et al. [2023]). Strategies to implement alternative uptake systems may further improve production in strains lacking Gcd. This would be widely applicable in many different cultivation approaches that use glucose as substrate.

Native energy-driven glucose transport systems are often beneficial in environments with low carbohydrate concentration because they allow uptake with high affinity (Jeckelmann and Erni [2020]). This can be a distinct advantage under competitive conditions with limited glucose availability. In an artificial laboratory environment, substrates are usually supplied in concentrations exceeding the physiological K_M values of the respective uptake systems by several orders of magnitude. Therefore, the native sugar transport is usually not adapted to bioprocess requirements (Jeckelmann and Erni [2020]) and in the case of some pseudomonads, it leads to excessive acidification by gluconate as a carbon sequestration strategy (del Castillo et al. [2007]).

An alternative glucose uptake system, specifically interesting for biotechnological applications, can be found in *Zymomonas mobilis*. This bacterium naturally occurs in carbohydrate-rich environments and is an established facultative anaerobic host for ethanol fermentation with higher ethanol yields than yeasts due to its use of the Entner–Doudoroff pathway and limited biomass

formation (Rogers, Lee, and Tribe [1976]; Wang et al. [2018]; Yang et al. [2016]). In *Z. mobilis*, glucose is taken up along the concentration gradient through a glucose facilitator (Glf) protein without the expense of energy (Snoep et al. [1994]) unlike the glucose phosphotransferase system of *Escherichia coli* and the ABC glucose transporter of pseudomonads. The facilitated diffusion mediated by Glf_{Zm} enables glucose uptake at a very high specific uptake rate (Fuhrer, Fischer, and Sauer [2005]) but with a low reported affinity of K_M 1–4 mM (Parker et al. [1995]; Weisser et al. [1995]). Xylose and fructose (K_M 39 mM) are also transported, but with much lower affinities compared to glucose (Kurgan et al. [2021]; Weisser et al. [1995]). Glf_{Zm} appears as a promising transporter for sugars in an energy-independent manner that might be favourable in bioprocesses with high metabolic energy demands caused by, for example, osmotic stress or product toxicity. In *E. coli*, replacing the PEP-consuming phosphotransferase system with Glf_{Zm} increased shikimate production (Yi et al. [2003]), while the expression of Glf_{Zm} in *P. putida* enabled the use of alternative carbohydrates (Bujdoš et al. [2023]).

In this study, Glf_{Zm} was introduced into several genome-reduced *chassis* strains (GRC3) of *P. taiwanensis* VLB120. These strains were previously tailored for improved efficiency in bioprocesses by removal of dispensable or disadvantageous cellular features (Wynands et al. [2019]), which is expanded in this study by the engineering of glucose uptake. Its impact on production strains was investigated for the biosynthesis of resveratrol and cinnamate, showcasing the applicability of Glf_{Zm} and its benefits in *Pseudomonas* for the production of different molecules from glucose.

2 | Materials and Methods/Experimental Section

2.1 | Cultivation Conditions, Media, DNA Techniques

Escherichia coli and *Pseudomonas taiwanensis* VLB120 strains were cultured on agar plates (15 g L^{-1}) or in LB medium (10 g L^{-1} peptone, 5 g L^{-1} sodium chloride, and 5 g L^{-1} yeast extract) or modified mineral salts medium (MSM) with 3-fold concentration of buffer components (3×22.3 mM K$_2$HPO$_4$ and 3×13.6 mM NaH$_2$PO$_4$) (Hartmans et al. [1989]) at 37°C and 30°C, respectively. Growth experiments started from serial precultures, beginning in LB, followed by an adaptation culture in MSM and the following cultivation under experimental conditions with either 20 or 30 mM glucose. Antibiotics were added if required (50 mg L^{-1} kanamycin sulphate; 20 mg L^{-1} gentamicin sulphate solution; 100 mg L^{-1} ampicillin sodium salt; tetracycline hydrochloride 30 mg L^{-1}). For biotransformation experiments for the production of resveratrol, 1 mM *p*-coumarate was supplemented to the medium. Plasmids were constructed using the NEBuilder HiFi DNA Assembly methodology, and knockout procedures were performed using the I-SceI system that is based on two consecutive homologous recombination events as described previously (Schwanemann et al. [2023]; Wynands et al. [2018]). All strains, plasmids and DNA oligonucleotides used in this study are shown in Tables [S1], [S2] and [S3], respectively. The amino acid sequence of the glucose facilitator protein from *Zymomonas mobilis* (Glf_{Zm}) used for codon optimization corresponds to UniProt entry P21906 (Table [S4]). The 200 bp upstream of *gtsABCD*
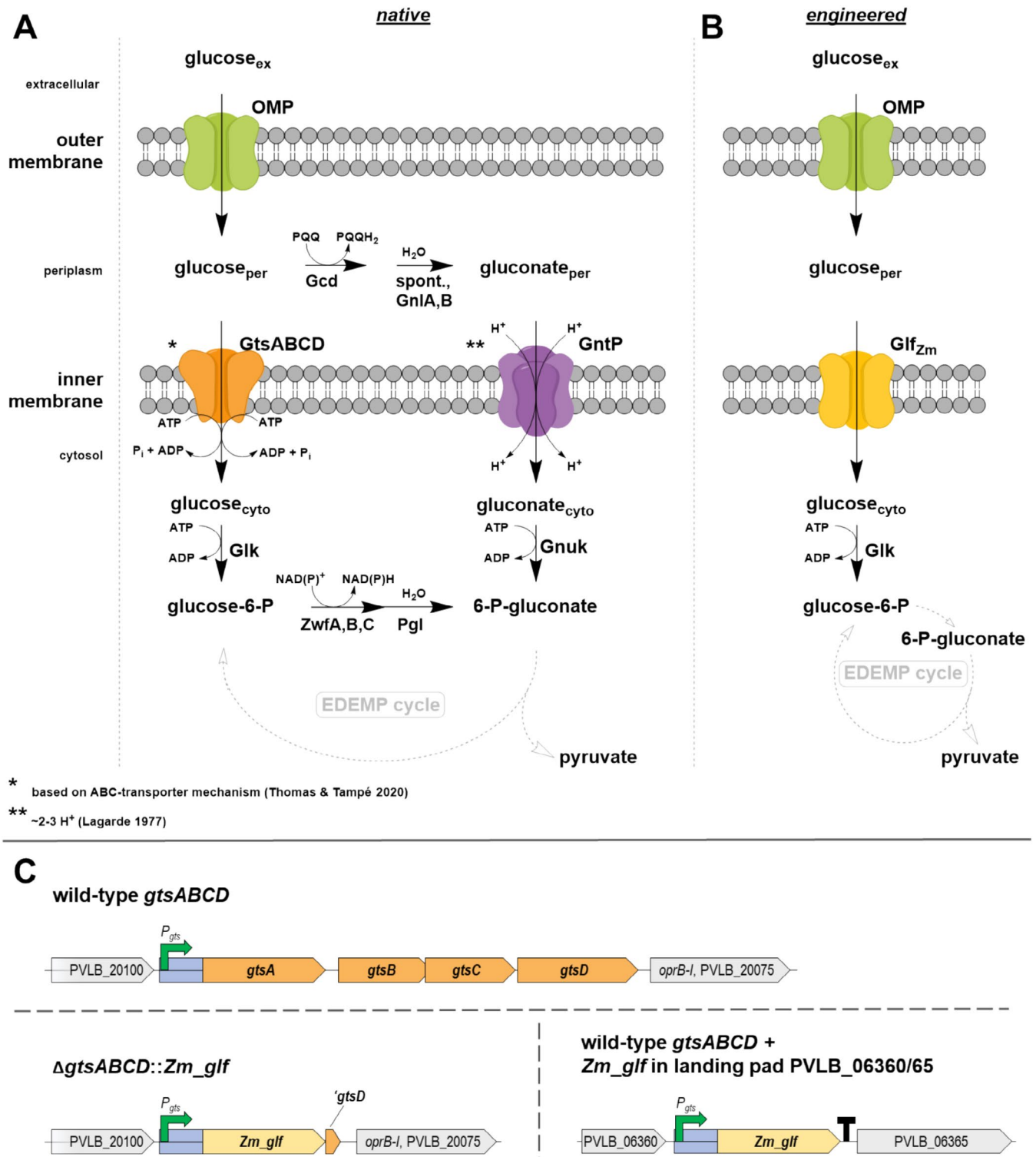
FIGURE 1 | Engineered glucose uptake in *P. taiwanensis*. (A) Overview of native and (B) engineered glucose transporters in *P. taiwanensis* VLB120. Energetic drivers are illustrated according to the mechanism of transport indicated by asterisks. (C) Genetic organisation of glucose uptake-encoding genes in wild-type and engineered *P. taiwanensis* VLB120. '*gtsD*, remaining 32 bp of truncated coding sequence of native GtsD; EDEMP cycle, Entner–Doudoroff-Embden-Meyerhof-Parnas-pentose phosphate cycle; Gcd, PQQ-dependent glucose dehydrogenase; Glf$_{Zm}$, glucose facilitator protein from *Zymomonas mobilis*; Glk, glucokinase; Gnl, gluconolactonase; GnuK, D-gluconate kinase; GtsABCD, glucose ABC transporter; GntP, D-gluconate transporter; OMP, outer membrane porins; *oprB-I*, coding sequence of a carbohydrate-selective porin; Pgl, 6-phosphogluconolactonase; P_{gts}, presumed promoter region (200 bp upstream of the start codon of *gtsA* PVLB_20095–80); PQQ, pyrroloquinoline quinone; Zwf, Glucose-6-phosphate dehydrogenase (Nikel et al. 2015).

(PVLB_20095–80) was considered as the promoter region of heterologous Glf_{Zm} expression constructs.

Cultivations for the production of cinnamate (Otto et al. 2019) or resveratrol were performed in a 24-square well plate system Duetz as described previously by Schwanemann et al. (2023).

2.2 | Analytical Methods

Growth characterisation experiments were performed in 96-square well plates in the Growth Profiler 960 with respective calibration for conversion of 'green values' from pixels of a picture into OD_{600} equivalents.

Determination of the optical density was performed at 600 nm with GE Healthcare Ultrospec 10 device from Fischer Scientific GmbH (Schwerte, Germany).

To determine biomass concentration after 24 h by cell dry weight (CDW) and OD_{600}, experiments were executed in 100 mL shake flasks with 11% filling volume, and samples of 10 mL were collected in dried and pre-weighted glass centrifuge tubes from Glaswarenfabrik Karl Hecht GmbH & Co KG (Sondheim, Germany) that were centrifuged for 20 min at 4000 g and washed with 5 mL of a 0.9% (w/v) sodium chloride solution. After discarding the supernatant, the pellets were dried at 65°C. A respective medium control was processed in parallel.

For the analysis of resveratrol, 1 mL culture broth was extracted with ethyl acetate and processed further in amber glass vials, as described in detail previously (Schwanemann et al. 2023). Cinnamate and p-coumarate were quantified from filtered culture supernatant, and all supplemented precursors and products were analysed by HPLC.

For the detection and quantification of cinnamate, p-coumarate and resveratrol, a 1260 Infinity II HPLC with a 1260 DAD WR (Agilent Technologies) and an ISAspher 100-5 C18 BDS column (Isera, Düren, Germany) was used, utilising the identical settings and gradients of 0.1% trifluoroacetic acid and acetonitrile as previously for resveratrol analysis (Schwanemann et al. 2023). Cinnamate, p-coumarate and resveratrol were measured at 245, 280, and 310 nm and eluted after 11.54, 7.13, and 9.08 min, respectively.

All experiments were executed in replicates, and significance analysis was performed using 1-way ANOVA with a confidence interval of $p < 0.05$.

3 | Results and Discussion

3.1 | Effect of Glf_{Zm} on the Growth of *P. taiwanensis*

The expression of *Zm_glf*, encoding the glucose facilitator of *Z. mobilis*, in different *P. taiwanensis* GRC3 strains was achieved either through the exchange of glucose transporter genes *gtsABCD* with *Zm_glf* or by the additional expression of *Zm_glf* from a neutral genomic landing pad (PVLB_06360-65)

(Köbbing et al. 2024) (Figures 1B and 2). The same modifications were introduced in GRC3Δ*gcd* and a malonyl-CoA platform strain (GRC3Δ6 MC-III) (Schwanemann et al. 2023) which are incapable of periplasmic glucose oxidation to gluconate. To elucidate the effects of these modifications, final biomass concentrations were determined by CDW to calculate biomass yields (Figure 2A) and growth rates were determined by OD_{600} equivalent from Growth Profiler experiments (Figure 2B). The transporter exchange did not alter biomass yields in the GRC3 background. The additional expression of *Zm_glf* even reduced the biomass yield compared to the GRC3 control and the GRC3 strain with the replaced transporter. However, the difference was only significant when compared to the latter. In GRC3Δ*gcd* strain background, a similar trend of final biomass yields was observed for additional Glf_{Zm} expression. The reduced biomass yield might be an effect of limited membrane space (Daddaoua et al. 2017; Nerke et al. 2024) and increased maintenance when *Zm_glf* is additionally expressed from the same promoter as native *gtsABCD*.

Upon expression of *Zm_glf* in strain GRC3Δ6 MC-III, which was metabolically engineered for increased malonyl-CoA supply including a *gcd* deficiency (Schwanemann et al. 2023), no significant changes were determined regarding biomass yield, but a reduced growth rate was observed. A trend to yield a high final biomass for the exchanged transporter strain was noted, although the respective control reached a slightly lower final biomass than the GRC3 control strains. That might not be surprising given that strain MC-III was engineered for bioproduction.

When comparing growth rates of the respective strains (Figures 2B, S5) the deletion of Gcd alone did not decrease the maximal growth rate, but the malonyl-CoA platform strain, GRC3Δ6 MC-III, has a 24% decreased growth rate ($0.36 \, h^{-1}$) compared to the GRC3 ($0.47 \, h^{-1}$). Strains with intact periplasmic gluconate formation showed no impact on their growth rate compared to the parental strain. However, strains with deleted Gcd and GtsABCD replaced by Glf_{Zm}, in which glucose is taken up solely via the heterologous Glf_{Zm}, showed a severe growth rate reduction of approximately half. The additional expression of Glf_{Zm} decreased the rate by 23% or 19% for strains lacking only Gcd (GRC3Δ*gcd*) or the malonyl-CoA platform strain (GRC3Δ6 MC-III), respectively.

In general, it can be concluded that the glucose uptake systems in *P. taiwanensis* VLB120 can be replaced by Glf_{Zm} with little effect on biomass yield, but the growth rate is significantly affected in engineered strains that rely solely on glucose import through the Glf_{Zm} transporter. Since *Zm_glf* evolved in a different host and under different metabolic conditions at high carbohydrate concentrations, it is likely unbalanced, interfering with the host's cell envelope and expression capabilities, which could be addressed and optimised by adaptive laboratory evolution (ALE). However, the effects of glucose uptake modifications on growth were only tested on non-producing strains. Exponentially growing *Pseudomonas*, especially genome-reduced GRC3 with higher energy efficiency (Wynands et al. 2019), is not energy limited (Zobel et al. 2017). Deep engineering for the conversion of glucose to products typically causes growth rate reductions and higher energy demands, and here the use of Glf_{Zm} might be more beneficial.
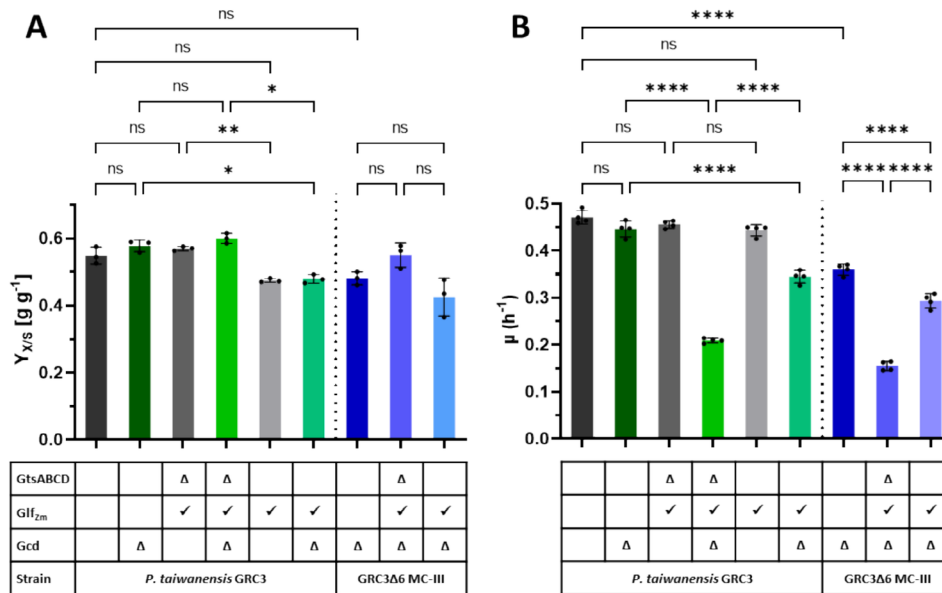
FIGURE 2 | Biomass yields and kinetics of different Glf_{Zm} strains. (A) Final biomass yields ($g_{biomass}/g_{glucose}$) resulting from the determined cell dry weight of *P. taiwanensis* VLB120 GRC3, GRC3Δgcd and GRC3Δ6MC-III with either replaced glucose transporter gene *gtsABCD* by *Zm_glf* indicated by Δ for GtsABCD or with *Zm_glf* expression from landing pad PVLB_06360/65. Grown in shaken-flask cultures with 30 mM (5.4 g L^{-1}) glucose and 3-fold buffered MSM for 24 h with inoculation of 1% (*v/v*) from the adaption culture. (B) Growth rate of the same strains determined in Growth Profiler experiment from OD_{600} equivalent values obtained in 3-fold buffered MSM with 20 mM glucose (Figure S5). Error bars represent the standard deviation ($n = 3$ in A or $n = 4$ in B). Statistical analysis was made by 1-way ANOVA (ns, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ****, $p < 0.0001$). Ns, not significant; $Y_{X/S}$, biomass yield; μ, growth rate.

## 3.2 | Effect of Glf_{Zm} on Cinnamate Formation by Engineered *P. taiwanensis*

To test the effect of Glf_{Zm} in *P. taiwanensis* VLB120 aromatics production strains, a high-yield cinnamate producer was equipped with the alternative glucose uptake system. Strain *P. taiwanensis* GRC3Δ8$\Delta pykA$-tap (Otto et al. 2019) (here called GRC3 PHE) was the basis for the phenylalanine platform strain. When equipped with a phenylalanine ammonia-lyase (AtPAL2) from *Arabidopsis thaliana* at the genomic Tn7 integration site (*attTn7::P_{14f}-AtPAL2*), this strain produces cinnamate from glucose with a yield of 23% C-mol C-mol^{-1} (Otto et al. 2019).

Cinnamate biosynthesis was evaluated in engineered strains featuring either native or modified glucose transport, as well as with and without periplasmic oxidation of glucose to gluconate (Figure 3). In strains with exchanged glucose uptake system, titres were significantly improved by 5% to 4.3 ± 0.03 mM. Further, a 15% increase to 4.7 ± 0.03 mM was observed in the production strain with additional expression of *Zm_glf*. In this strain, the expression driven by the P_{gts} promoter may compete with native GtsABCD expression and gluconate uptake. Deletion of *gcd* slightly reduced final OD_{600} and had a minor but significant negative effect on cinnamate titres (4.1 ± 0.04 mM for GRC3 PHE, 4.0 ± 0.03 mM GRC3 PHE Δgcd). This relatively minor effect on production would likely be offset in scaled-up batch cultures through the avoidance of transient acid formation. Without periplasmic Gcd, both expression strategies for Glf_{Zm} improved cinnamate titres to approximately 4.4 mM from 30 mM glucose compared to strain

GRC3 PHE Δgcd, which constitutes a significant 10% improvement in production.

3.3 | Effect of Glf_{Zm} on Resveratrol Formation in *P. taiwanensis*

High-yield cinnamate production poses a high drain on central metabolites with a relatively non-toxic product. In contrast, secondary metabolites are typically produced at lower yields and titres, but their production can pose a higher stress on the cell in terms of metabolic burden. To differentiate between these effects, a malonyl-CoA platform strain (GRC3Δ6 MC-III) was evaluated for its ability to produce resveratrol from glucose and *p*-coumarate (Figure 4) with implemented *Zm_glf* modifications. Resveratrol production was enabled by equipping the strain with the corresponding stilbene production module (*attTn7::FRT-P_{14f}-his.AhSTS-Sc4CL^{A294G}*). The GRC3Δ6 MC-III control with the stilbene module produced 77.6 mg L^{-1} (0.34 mM) resveratrol from 30 mM glucose and 1 mM *p*-coumarate with 0.72 mM remaining precursor. This resveratrol production is in a similar range to those previously reported (Schwanemann et al. 2023). By exchanging the GtsABCD glucose transporter with Glf_{Zm}, resveratrol titres were increased to 97.7 mg L^{-1} (0.43 mM), which represents a 26% improvement and a yield of 18.1 $mg_{resveratrol}$ $g_{glucose}$ $^{-1}$. Since this strain background already features a *gcd* knockout, this constitutes a complete replacement of glucose uptake by Glf_{Zm}. In contrast to previous reports (Braga et al. 2018), no product loss was observed (Figure S6), with only 0.54 mM *p*-coumarate remaining. The additional expression of *Zm_glf* in a GtsABCD$^+$ background reduced overall biomass and resveratrol titre (Figure 4).
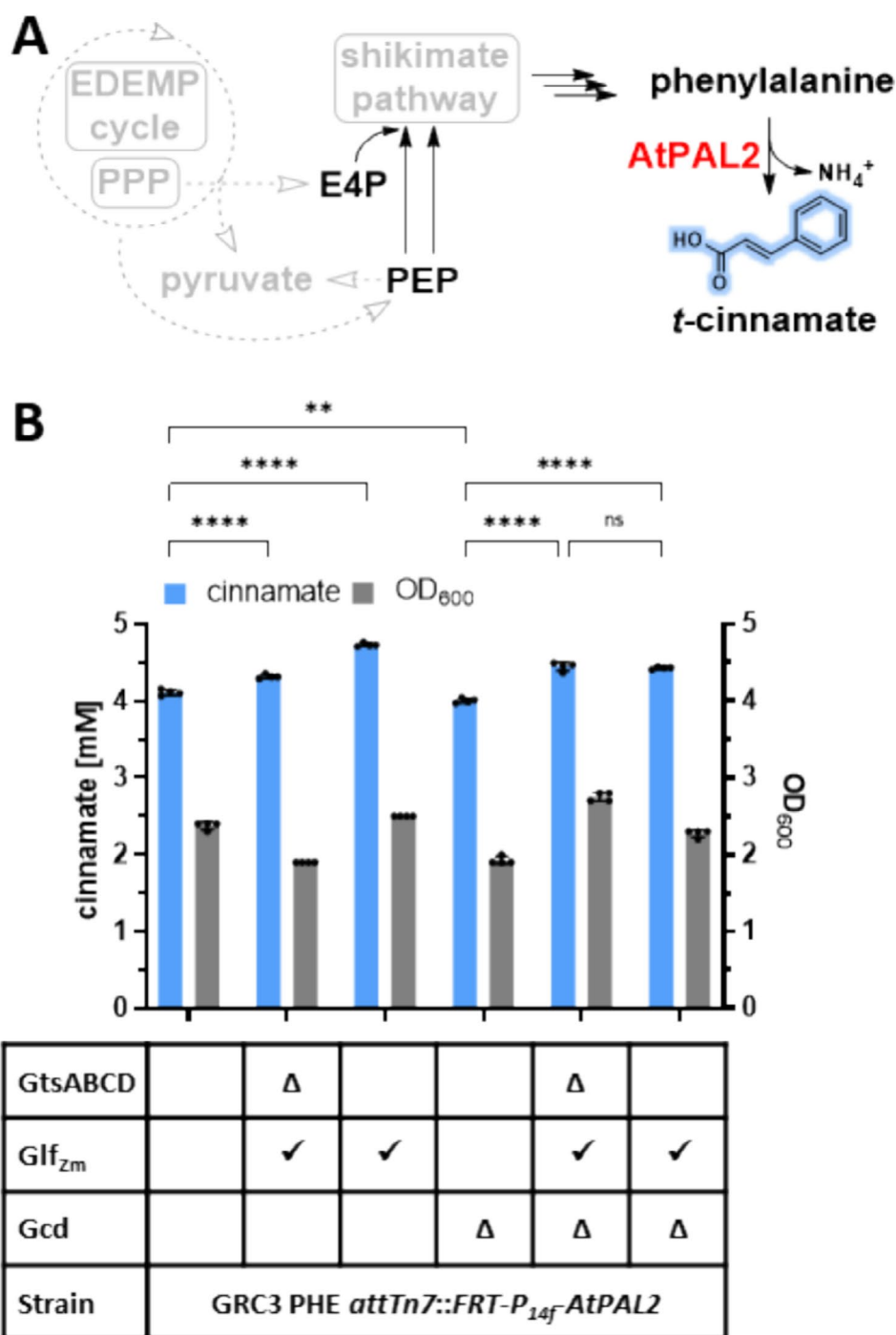
FIGURE 3 | (A) Schematic overview of cinnamate biosynthesis and (B) cinnamate production by different Glf_{Zm} strains. Cinnamate titre and OD_{600} of GRC3 PHE and GRC3 PHE Δgcd with markerless cinnamate production module ($attTn7$::FRT-P_{14f}-$AtPAL2$) with either replaced glucose transporter genes $gtsABCD$ by Zm_glf or with Zm_glf expression from landing pad PVLB_06360/65. Strains were grown in 24-square deep well plate with 30 mM (5.4 g L^{-1}) glucose, 3-fold buffered MSM, sampled after 115 h in stationary phase. Error bars represent the standard deviation ($n=4$). Statistical analysis was made by 2-way ANOVA (ns, $p > 0.05$; **, $p < 0.01$; ****, $p < 0.0001$). E4P, erythrose-4-phoasphate; EDEMP cycle, Entner-Doudoroff-Embden-Meyerhof-Parnas-pentose phosphate cycle; PEP, phosphoenolpyruvate; PPP, pentose phosphate pathway.

Consequently, GRC3Δ6 MC-III Δ$gtsABCD$::Zm_glf with stilbene production module was identified as a better platform for resveratrol production from p-coumarate than GRC3Δ6 MC-III in spite of its reduced growth rate (Figure 2) and thus strain $P.$ $taiwanensis$ GRC3Δ6MC-III Δ$gtsABCD$::Zm_glf is hereafter called GRC3Δ6MC-IV (generation No. 4 of the malonyl-CoA platform strain).

4 | Conclusion

In this study, we investigated the impact of modified glucose uptake by a passive facilitator in $P.$ $taiwanensis$ VLB120 GRC3 and derivatives thereof. Final biomass yields of GRC3 and Gcd-deficient variants were not affected significantly when $gtsABCD$ was replaced by Glf_{Zm}. Additional expression of Glf_{Zm} did not
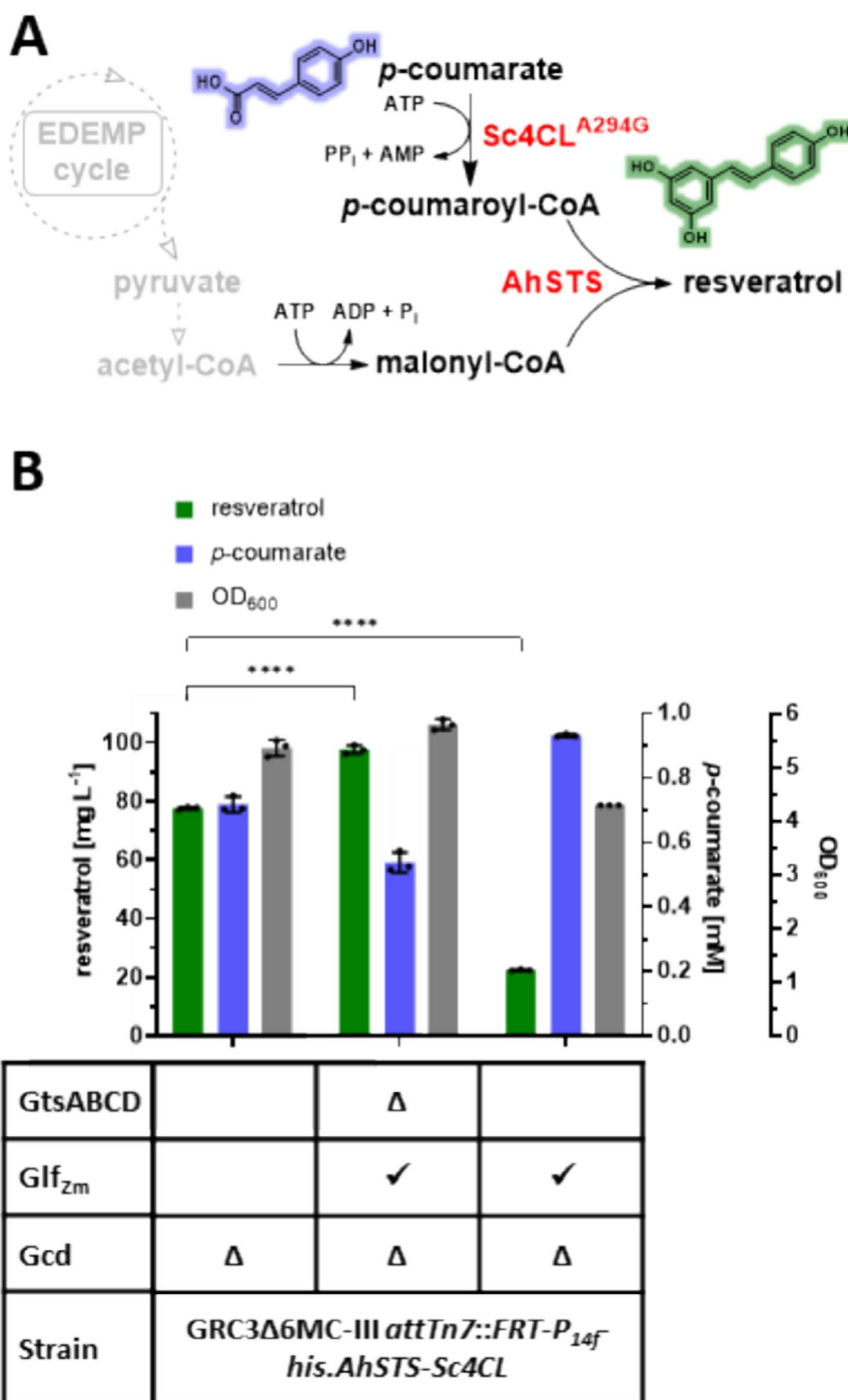
FIGURE 4 | (A) Schematic overview of resveratrol biosynthesis and (B) resveratrol production by different Glf_{Zm} strains. Resveratrol and *p*-coumarate titre of GRC3Δ6MC-III with stilbene module ($attTn7::FRT-P_{14f}-his.AhSTS-Sc4CL^{A294G}$) with either replaced glucose transporter gene *gtsABCD* by *Zm_glf* or with additional *Zm_glf* expression from landing pad PVLB_06360/65. Grown in 24-square deep well plate with 30 mM (5.4 g L^{-1}) glucose, 3-fold buffered MSM and 1 mM *p*-coumarate for 24 h. Error bars represent the standard deviation ($n=3$). Statistical analysis was made by 2-way ANOVA (****, $p < 0.0001$). EDEMP cycle, Entner-Doudoroff-Embden-Meyerhof-Parnas-pentose phosphate cycle.

necessarily lead to improved growth on glucose or production, indicating that glucose uptake is not limited by transport *per se* or that membrane stress from the additional transport protein outweighed any potential benefit. In the absence of production, the resource-efficient passive glucose uptake came at the cost of reduced growth rates when both native glucose uptake and periplasmic oxidation were eliminated. However, this replacement boosted bioproduction of value-added aromatic molecules from

different primary pathways in strains that already were highly optimised, highlighting the strategy's applicability, especially under demanding biotechnological conditions. The enhancement was observed not only in the synthesis of cinnamate (10% increase), a shikimate pathway-derived product, but also in resveratrol production (26% increase), which is derived from malonyl-CoA and supplemented p-coumarate. Hereby Glf_{Zm} can demonstrate its benefits in the absence of Gcd when the used host relies on cytosolic glucose metabolism, which is used in several bioprocess setups to ensure pH stability. However, the implementation of Glf_{Zm} in production strains with native glucose oxidation can be improved as well, as demonstrated for cinnamate formation. This straightforward engineering of the strain could boost future efforts in optimising *Pseudomonas* for production applications.

Author Contributions

Tobias Schwanemann: conceptualization, methodology, validation, formal analysis, investigation, funding acquisition, writing – original draft, visualization. **Nicolas Krink:** conceptualization, writing – review and editing, supervision. **Pablo I. Nikel:** conceptualization, resources. **Benedikt Wynands:** validation, writing – review and editing, supervision. **Nick Wierckx:** conceptualization, validation, resources, writing – review and editing, supervision, funding acquisition, project administration.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.