Role of L-alanine for redox self-sufficient amination of alcohols

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Abstract

Background: In white biotechnology biocatalysis represents a key technology for chemical functionalization of non-natural compounds. The plasmid-born overproduction of an alcohol dehydrogenase, an L-alanine-dependent transaminase and an alanine dehydrogenase allows for redox self-sufficient amination of alcohols in whole cell biotransformation. Here, conditions to optimize the whole cell biocatalyst presented in (Bioorg Med Chem 22:5578–5585, 2014), and the role of L-alanine for efficient amine functionalization of 1,10-decanediol to 1,10-diaminodecane were analyzed.

Results: The enzymes of the cascade for amine functionalization of alcohols were characterized in vitro to find optimal conditions for an efficient process. Transaminase from Chromobacterium violaceum, TaCv, showed three-fold higher catalytic efficiency than transaminase from Vibrio fluvialis, TaVf, and improved production at 37°C. At 42°C, TaCv was more active, which matched thermostable alcohol dehydrogenase and alanine dehydrogenase and improved the 1,10-diaminodecane production rate four-fold. To study the role of L-alanine in the whole cell biotransformation, the L-alanine concentration was varied and 1,10-diaminodecane formation tested with constant 10 mM 1,10-decanediol and 100 mM NH₄Cl. Only 5.6% diamine product were observed without added L-alanine. L-alanine concentrations equimolar to that of the alcohol enabled for 94% product formation but higher L-alanine concentrations allowed for 100% product formation. L-alanine was consumed by the E. coli biocatalyst, presumably due to pyruvate catabolism since up to 16 mM acetate accumulated. Biotransformation employing E. coli strain YYC202/pTrc99a-ald-adh-taCv, which is unable to catabolize pyruvate, resulted in conversion with a selectivity of 42 mol-%. Biotransformation with E. coli strains only lacking pyruvate oxidase PoxB showed similar reduced amination of 1,10-decanediol indicating that oxidative decarboxylation of pyruvate to acetate by PoxB is primarily responsible for pyruvate catabolism during redox self-sufficient amination of alcohols using this whole cell biocatalyst.

Conclusion: The replacement of the transaminase TaVf by TaCv, which showed higher activity at 42°C, in the artificial operon ald-adh-ta improved amination of alcohols in whole cell biotransformation. The addition of L-alanine, which was consumed by E. coli via pyruvate catabolism, was required for 100% product formation possibly by providing maintenance energy. Metabolic engineering revealed that pyruvate catabolism occurred primarily via oxidative decarboxylation to acetate by PoxB under the chosen biotransformation conditions.

Keywords: Redox self-sufficient amination, Whole cell biotransformation, Escherichia coli, Transaminase, Chromobacterium violaceum, Energy maintenance, Acetate formation, Pyruvate oxidase, Phosphate acetyltransferase, Acetate kinase

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Background

White biotechnology is the key technology for alternative and sustainable production of e.g. fine chemicals. Its application in biocatalysis is considered a branch of Green Chemistry which can replace or complement routes of chemical modification and functionalization. Enzymes catalyze reactions under mild conditions contrarily to chemical catalysts which often demands high pressure and temperature as well as toxic solvents. Among others, amine functionalization of chemical compounds is an important approach in biocatalysis to produce (poly)amines which are components of for example synthetics and coatings. This can be performed by amino acid dehydrogenases catalyzing NADH-dependent reductive amination of oxo-acids with ammonium or by transaminases transferring an amino group from a donor amine to a carbonyl compound. The cofactor pyridoxal-phosphate is covalently bound to the catalytic center of α-transaminases to transfer the amino group to the acceptor molecule [1,2].

Generally, in biocatalysis the use of a transaminase instead of an amino-acid dehydrogenase offers the access to a wider range of substrates since amino-acid dehydrogenases accept a restricted spectrum of α-keto acids. Coupling of both types of enzymes enables amine functionalization of a wider range of carbonyl compounds from ammonium in a redox dependent manner. Therefore, redox cofactor recycling is important to reduce the process costs. Cofactor recycling by cascading with, for example, glucose dehydrogenase or formate dehydrogenase or for whole cell biocatalysts with cellular glucose catabolism have been developed [3,4]. A three enzyme cascade for recycling redox cofactor and amino group donor has been demonstrated in cell free biocatalysis using an alanine dehydrogenase coupled with a L-alanine-dependent transaminase and an alcohol dehydrogenase [5]. The initial alcohol oxidation by an alcohol dehydrogenase yields NADH and the aldehyde which is converted to the amine by L-alanine-dependent transamination (Figure 1A). Alanine dehydrogenase recycles L-alanine using ammonium and NADH releasing NAD as redox cofactor for the alcohol dehydrogenase. Recently, this concept of redox self-sufficient amination of alcohols (Figure 1A) was realized in a whole cell process with Escherichia coli by plasmid-born overproduction of the alcohol dehydrogenase of Bacillus stearothermophilus, the transaminase of Vibrio fluvialis and the alanine dehydrogenase of Bacillus subtilis [6]. Advantageously, the whole cell process only required alcohol, L-alanine and ammonium but neither NAD nor PLP. In principle, L-alanine addition is not required in the three-enzyme-cascade and amination of alcohols to amines should be possible with an ammonium salt. However, whole cells require energy to maintain viability as well as for transport processes e.g. substrate uptake and product export as well as protein synthesis under starvation or other stress conditions. Since non-growing cells are typically used for whole cell biotransformation, the non-growth-associated maintenance coefficient reflects the cell’s requirement to catalyzebicate an energy substrate such as glucose to keep the cell’s viability and functionality. Therefore, the role of L-alanine in the whole cell biotransformation of alcohols to amines was analysed and conditions for optimizing the whole cell biocatalyst towards improved amino functionalization of alcohols were tested.

Results

Construction of the whole cell biocatalyst W3110/pTrc99A-ald-adh-tacVf and its comparison to W3110/pTrc99A-ald-adh-tacVf in vitro and in vivo

The whole cell biocatalyst W3110/pTrc99A-ald-adh-tacVf was previously shown to enable redox self-sufficient amination of a variety of alcohols [6] and involved thermo-sensitive transaminase from Vibrio fluvialis. Due to the thermostable alcohol dehydrogenase of B. stearothermophilus an increased reaction temperature for redox self-sufficient amination was considered to improve the production rate. Therefore, the gene for the transaminase of V. fluvialis was replaced by the gene for the transaminase of C. violaceum in the IPTG-inducible vector pTrc99A-ald-adh-tacVf. The vector was used to transform E. coli W3110 to yield the whole cell biocatalyst W3110/pTrc99A-ald-adh-tacCv. Enzyme activity assays of the newly constructed whole cell biocatalyst revealed that all three genes were functionally expressed. The crude extracts displayed enzyme activities of 9.8 ± 1.1 and 0.55 ± 0.03 U/mg for the alanine dehydrogenase and the alcohol dehydrogenase, respectively, which was similar to the activities in W3110/pTrc99a-ald-adh-tacVf [6]. However, with (S)-(−)α-methylbenzylamine as substrate, the specific activities of transaminase TacCv of 0.62 ± 0.01 in W3110/pTrc99a-ald-adh-tacCv were two-fold higher than that of TacVf in W3110/pTrc99a-ald-adh-tacVf.

The catalytic efficiencies of both transaminases were estimated with L-alanine as donor substrate and hexanal as acceptor substrate. Therefore, crude extracts of W3110/pTrc99a-tacVf and W3110/pTrc99a-tacCv were assayed in a 50 mM potassium-phosphate buffer pH 7.4 with constant hexanal and varying L-alanine concentrations at 37°C. The L-alanine-dependent transamination of hexanal to hexylamine could be detected for both transaminases and Km values and Vmax-values for TacVf were 20 mM L-alanine and 0.3 U/mg, respectively, and 35 mM L-alanine and 2 U/mg, respectively, for TacCv (Table 1). The catalytic efficiency reflected by the Vmax/Km-value was 3-fold higher for TacCv than for TacVf (0.06 compared to 0.02 U mg⁻¹ M⁻¹).

To compare the whole cell biocatalysts W3110/pTrc99a-ald-adh-tacCv and W3110/pTrc99a-ald-adh-tacVf for the redox-self-sufficient amination of alcohols conversion of the diol 1,10-decanediol was tested. The addition of 1,10-decanediol, 100 mM L-alanine and 100 mM...
NH₄Cl resulted in 100% product formation employing the newly derived catalyst W3110/pTrc99a-ald-adh-ta Cv (Table 2) as well as employing the previously designed W3110/pTrc99a-ald-adh-ta Vf [6]. When the L-alanine concentration was reduced to 50 mM or 20 mM, production of 1,10-diaminodecane using W3110/pTrc99a-ald-adh-ta Cv and W3110/pTrc99a-ald-adh-ta Vf was reduced to 84% and 86%, respectively, and 73% and 50%, respectively (Table 2). HPLC analysis revealed that both strains consumed between 16 and 30 mM L-alanine (Table 2). Taken together, the newly constructed biocatalyst W3110/pTrc99a-ald-adh-ta Cv showed higher activity and catalytic efficiency with respect to the transaminase and higher selectivity at 37°C and with low L-alanine concentrations.

### Table 1: In vitro estimation of the catalytic efficiencies of transaminases Ta Vf and Ta Cv with L-alanine and hexanal as substrates

| Origin of the transaminase | Km for L-alanine [mM] | Vmax [U/mg] | Vmax/Km |
|---------------------------|----------------------|-------------|---------|
| V. fluvialis              | 20.00 ± 1.10         | 0.30 ± 0.01 | 0.02    |
| C. violaceum             | 35.00 ± 2.20         | 2.00 ± 0.07 | 0.06    |

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**Influence of the reaction temperature on amination of 1,10-decanediol to 1,10-diaminodecane by W3110/pTrc99a-ald-adh-ta Vf and W3110/pTrc99a-ald-adh-ta Cv.**

The three-enzyme-cascade contains thermostable alcohol dehydrogenase from the thermophilic *B. stea rodephilus*, but little is known about the activity of the alanine dehydrogenase from *B. subtilis* and the two transaminases of *V. fluvialis* and *C. violaceum* at higher temperatures. The activities of all enzymes were analyzed at varying temperatures in crude extracts of W3110/pTrc99a-ald, W3110/pTrc99A-ald, W3110/pTrc99a-ta Vf as well as W3110/pTrc99a-ta Cv. For the alanine dehydrogenase of *B. subtilis* and the alcohol dehydrogenase of *B. stea rodephilus*, enzyme activities increased with temperature ranging from 30°C to 60°C and 20 U/mg and 1.8 U/mg, respectively, with maxima at about 60°C (Figure 2). The activity of the
transaminase of *V. fluvialis* (Ta<sub>Vf</sub>) in the crude extract of W3110/pTrc99a-ald-adh-ta<sub>Vf</sub> dropped from 0.5 U/mg to 0.03 U/mg when the reaction temperature increased from 37°C to 42°C. The activity of transaminase of *C. violaceum* (Ta<sub>Cv</sub>) was also lower at 42°C (0.13 U/mg) than at 37°C (0.66 U/mg), but its activity at 42°C was four-fold higher than that of Ta<sub>Vf</sub> at 42°C (Figure 2).

To assay the influence of the reaction temperature on the amination cascade, whole cell biotransformations with W3110/pTrc99a-ald-adh-ta<sub>Vf</sub> and W3110/pTrc99a-ald-adh-ta<sub>Cv</sub> were performed at 37°C, 40°C and 42°C being suitable temperatures for the host *E. coli*. 1,10-decanediol was chosen as a substrate since it was among the best substrates of this cascade [5,7] and, moreover, the use of this dialcohol allows for monitoring 1-amino-10-decanol, an intermediate of diamine formation. The diamine production of both strains was compared in a resting buffer system using 1,10-decanediol as substrate, 100 mM L-alanine and 100 mM NH₄Cl. In vivo amination of 1,10-decanediol performed by the two whole cell biocatalysts reached 100 mol-% selectivity after 24 hours at 37°C and production rates of about 0.06 g/g*h (0.35 mmole/g*h) were calculated (Figure 3). Furthermore, the increase of the reaction temperature from 37°C to 40°C and 42°C improved the production rates to 0.15 g/g*h (0.87 mmole/g*h) and 0.2 g/g*h (1.16 mmole/g*h), respectively, when the newly constructed W3110/pTrc99a-ald-adh-ta<sub>Cv</sub> was employed. Contrarily, with W3110/pTrc99a-ald-adh-ta<sub>Vf</sub> substrate conversion to 1,10-diaminodecane (74% and 56%, respectively) and production rates (0.04 g/g*h and 0.03 g/g*h, respectively) were lower at 40°C and 42°C, respectively, as compared to 37°C (Figure 3). Thus, under the chosen conditions W3110/pTrc99a-ald-adh-ta<sub>Cv</sub> was superior to W3110/pTrc99a-ald-adh-ta<sub>Vf</sub> at an elevated temperature.

The role of L-alanine for the redox self-sufficient amination of alcohols in a whole cell process

L-alanine was a component of the hitherto described redox self-sufficient amination of alcohols with cascaded enzymes or as whole cell biotransformation [5,6] as well as in the experiments described above to serve as amino group donor in L-alanine-dependent transamination. However, conceptually redox self-sufficient amination of alcohols does not require the addition of L-alanine, but may proceed from ammonium only (Figure 1A). Thus, in order to find out if external addition of L-alanine is necessary to drive amination of 1,10-decanediol via this cascade, transaminase of *V. fluvialis* (Ta<sub>Vf</sub>) in the crude extract of W3110/pTrc99a-ald-adh-ta<sub>Vf</sub> dropped from 0.5 U/mg to 0.03 U/mg when the reaction temperature increased from 37°C to 42°C. The activity of transaminase of *C. violaceum* (Ta<sub>Cv</sub>) was also lower at 42°C (0.13 U/mg) than at 37°C (0.66 U/mg), but its activity at 42°C was four-fold higher than that of Ta<sub>Vf</sub> at 42°C (Figure 2).

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varying L-alanine concentrations were assayed using biocatalyst W3110/pTrc99a-ald-adh-taCv at 42°C with a constant concentration of 100 mM NH₄Cl. At reduced L-alanine concentrations of 50 mM and 20 mM complete conversion of 1,10-decanediol to 1,10-diaminodecane was observed after 6 hours. With 10 mM and 5 mM L-alanine selectivity was almost complete (94% and 93%, respectively), whereas only 5.6% conversion of 1,10-decanediol to 1,10-diaminodecane was observed without adding L-alanine (Table 3). Thus, L-alanine has to be present for efficient amination of alcohols using biocatalyst W3110/pTrc99a-ald-adh-taCv.

L-alanine added was utilized completely (at 5 and 10 mM) or partially (at 20 and 50 mM) during the whole cell biotransformation approach. Under certain conditions, acetate accumulated as by-product. When L-alanine was present at the same or lower concentrations as the substrate 1,10-decanediol, acetate accumulation was not observed, however, at higher L-alanine excess increasing

| Biotransformation conditions | Conversion [%] | L-alanine consumption [mM] | Acetate production [mM] |
|-----------------------------|----------------|---------------------------|------------------------|
| 1,10-decanediol | L-alanine | 100₄₄₀₄ | 70₄₄₀₄ | 70₄₄₀₄ |
| 10 mM | 50 mM | 100₄₄₀₄ | 23 | 16 |
| 10 mM | 20 mM | 100₄₄₀₄ | 13 | 3 |
| 10 mM | 10 mM | 94₄₄₀₄ | 10 | 0 |
| 10 mM | 5 mM | 93₄₄₀₄ | 5 | 0 |
| 10 mM | 0 mM | 5₄₄₀₄ | 0 | 0 |
| 0 mM | 20 mM | 0₄₄₀₄ | 20 | 2 |
| 10 mM | 0 mM +20 mM pyruvate | 70₄₄₀₄ | 7 | 7 |
acetate concentrations could be observed (Table 3). L-alanine (20 mM) was catabolized entirely in the absence of the substrate 1,10-decanediol. Pyruvate only partially replaced L-alanine since only 70% product formation were detected with 20 mM pyruvate. Taken together, these results indicate L-alanine consumption and acetate formation by the host’s central carbon metabolism.

To test if L-alanine consumption is important for the redox self-sufficient amination of alcohols with this whole cell biocatalyst or a dispensable side reaction, the host’s pyruvate catabolism was blocked at various positions. *E. coli* YYC202 cannot catabolize pyruvate (Figure 1) since it lacks the pyruvate dehydrogenase complex (deletion of aceEF), phosphoenolpyruvate synthetase (mutation of pps), pyruvate formate lyase (pflB) and pyruvate oxidase (poxB) [7]. Heterologous overexpression of *ald*, *adh* and *taCv* in *E. coli* YYC202 led to 42% conversion of 1,10-decanediol to 1,10-diaminodecane as compared to 100 mol-% selectivity obtained with the reference strain MG1655/pTrc99a-ald-adh-taCv (Figure 4). Since there are multiple metabolic blocks in *E. coli* YYC202, additional strains were analysed to identify which of the reactions blocked in *E. coli* YYC202 is most important for pyruvate catabolism. It is generally believed that pyruvate dehydrogenase complex (PDHC) is important under aerobic conditions and pyruvate formate lyase (PFL) under anoxic conditions while PoxB might be important during the transition between exponential and stationary phase since under microaerobic conditions both PDHC and PFL function poorly [8]. PoxB also contributes to aerobic growth especially at low grow rates [9]. It is known that the pyruvate oxidase gene *poxB* is induced in the stationary growth phase and this induction is dependent on the alternative sigma factor RpoS [10]. Thus, PoxB might be relevant under the conditions of whole cell biotransformation that are characterized by slow growth and limited oxygen. Therefore, *poxB* was deleted in *E. coli* W3110 (s. Materials and method) and the resulting mutant was transformed with pTrc99a-ald-adh-taCv. The whole cell biocatalyst lacking only *poxB*, W3110ΔpoxB/pTrc99a-ald-adh-taCv, showed about two-fold reduced amination of 1-10-decanediol to 1,10-diaminodecane (Figure 4) indicating that PoxB is the most important enzyme for pyruvate catabolism under the chosen biotransformation conditions. Moreover, since *E. coli* strain differences have been observed to affect biotransformation [11], which may be relevant even between the closely related strains W3110 and MG1655 used here [12], the effect of the lack of PoxB was also analysed in a different strain background, namely BW25113. Indeed, *E. coli* BW25113/pTrc99a-ald-adh-taCv converted about three times more (56%) 1,10-decanediol to 1,10-diaminodecane than its *poxB* mutant (16%; Figure 4). Taken together, the addition of L-alanine and its catabolism via pyruvate and pyruvate oxidase PoxB is important for efficient amino functionalization of alcohols by the described whole cell biocatalysts.

**Discussion**

Redox self-sufficient amination of alcohols by whole cell biotransformation benefitted from replacing the transaminase from *V. fluvialis* used previously [6] by transaminase *TaCv* from *C. violaceum* [13] since it showed higher activity at 42°C. Moreover, it showed higher catalytic efficiency with L-alanine as substrate (Table 1). The low activities of transaminases at 42°C appeared to be limiting the efficiency of the whole cell biocatalyst since alcohol dehydrogenase of *B. stearothermophilus* and L-alanine dehydrogenase are rather thermostable (Figure 2) [14,15]. Indeed, *TaCv*, which showed higher activity at 42°C than *TaVf*, allowed for efficient conversion of 1,10-decanediol to 1,10-diaminodecane at 42°C. Shifting the biotransformation temperature from 37°C to 42°C led to about three-fold faster conversion employing W3110/pTrc99a-ald-adh-taCv (Figure 3). Since at 37°C both W3110/pTrc99a-ald-adh-taCv and W3110/pTrc99a-ald-adh-taVf showed comparable production rates, the lower catalytic efficiency of *TaVf* did not limit product formation under these conditions.

Addition of L-alanine was required for full and fast conversion of 1,10-decanediol to 1,10-diaminodecane using the whole cell biocatalyst for alcohol amination. The
consumption of L-alanine over time suggested insufficient L-alanine recycling and loss of pyruvate via the cellular catabolism (Figure 1B; Table 3). This was less pronounced at higher 42°C possibly because under these conditions activities of both L-alanine dehydrogenase and alcohol dehydrogenase were increased allowing for more efficient redox cofactor recycling.

L-alanine served two functions in the biotransformation: As substrate in the transaminase reaction and to provide energy and reduction equivalents to the whole cell biocatalyst by catabolism of pyruvate, the co-product of L-alanine-dependent transamination (Figure 1). Under the non-growth conditions of whole cell biotransformation up to 20 mM of alanine were consumed (Table 3) with a rate of about 0.04 g/g*h, which is in the same order of magnitude as non-growth maintenance energy (0.055 to 0.07 g of glucose/g*h). Pyruvate addition only partially replaced L-alanine addition since product formation in the presence of 20 mM pyruvate was incomplete (70%; Table 3). In part, L-alanine was catabolized to acetate. If pyruvate is oxidatively decarboxylated to acetate by pyruvate oxidase PoxB, a reduction equivalent (ubiquinol) is formed which may be used (indirectly) for reductive amination by L-alanine dehydrogenase in the cascade. E. coli is known to produce acetate as overflow metabolite even under fully aerobic conditions, e.g. with excess glucose [16] when 10% - 30% of carbon flux is directed to acetate formation [17].

Acetate may be formed under aerobic growth conditions by the combined activities of pyruvate dehydrogenase complex PDHC, phosphotransacetylase Pta and acetate kinase AckA (Figure 1B). Besides the reduction equivalent NADH, this pathway yields ATP. A third pathway may be active as a mutant devoid of poxB, pta and ackA still produced acetate [18]. Fast catabolism of glucose to acetate followed by its reuse via acetyl-CoA synthetase (Figure 1B) may be advantageous in comparison to other microorganisms present in its natural habitat that slowly convert the limiting carbon source glucose [9]. In the absence of pyruvate oxidase PoxB conversion of 1,10-decanediol to 1,10-diaminododecane was reduced in about the same way as when PoxB and all other known enzymes for pyruvate degradation were missing, thus, indicating that PoxB is the major enzyme for pyruvate degradation under the chosen biotransformation conditions. In the biotransformation described here, the whole cell biocatalysts were harvested in the stationary phase when PoxB dominates.

Conclusions
The newly derived whole cell biocatalyst W3110/pTrc99a-ald-adh-taCV allowed for the improvement of redox self-sufficient amination of alcohols displayed by an increase in production rate. This was achieved by replacing the transaminase of V. fluvialis by the transaminase of C. violaceum, which showed higher activity at 42°C. The whole cell biocatalyst for redox self-sufficient amination of alcohols required L-alanine in concentrations equimolar to the dialcohol substrate for complete conversion to the diamine. L-alanine catabolism occurred primarily via pyruvate oxidase PoxB under the biotransformation conditions.

Materials and method
Bacterial strains, plasmids and oligonucleotides
The E. coli strains, plasmids and oligonucleotides used in this study are listed in Table 4.

Competent cells and vector cloning was performed according to standard DNA work procedure [21]. In this study two different cloning strategies for E. coli expression vectors based on IPTG-inducible pTrc99a were used. Firstly, cut sites were used for inserting a gene into a vector. Therefore, PCR-derived gene product ta of Chromobacterium violaceum [GI: 34105712; codon-optimized] (taCV_KpmIRBS_for; taCV_BamHI_rev) amplified by KOD Hot Start Polymerase Kit (Novagen) was cut with KpnI and BamHI and used for ligation with also KpnI and BamHI treated pTrc99A to generate pTrc99a-ald-taCV. To construct pTrc99a-ald-adh-taCV, the BamHI cut site was inserted upstream of taCV within the artificial operon ald-adh-taCV of pTrc99a-ald-adh-taCV by site directed mutagenesis using the oligonucleotides pTrc99a-ald-adh-taCV_mut_for [22]. The newly derived vector pTrc99a-ald-adh-taCV_mut was then cut by BamHI and ligated with BamHI cut ta of Chromobacterium violaceum that was amplified by KOD Hot Start Polymerase Kit (Novagen, pTrc99a-ald-adh-taCV _for, pTrc99a-ald-adh-taCV_rev). Secondly, to construct pTrc99A-ald-taCV, the gene ta of Vibrio fluvialis was amplified with the oligonucleotides taVf_RBS_for and taVf_rev and assembled with EcoI cut site inserts (Novagen) and Gibson assembly method [23]. Then, E. coli DH5α was transformed with the ligation products. CaCl2 competent E. coli DH5α were heat-shocked for the uptake of ligation products. Newly derived vectors were proven by sequencing and E. coli W3110 was transformed with correct plasmids pTrc99a-taNV, pTrc99a-taCV, and pTrc99a-ald-adh-taCV and E. coli MG1655 as well as E. coli YCC202 with pTrc99A-ald-adh-taCV.

Cultivation conditions and media
Standard cultivation of E. coli was performed in Luria-Bertani medium (LB-medium: 10 g/L NaCl, 10 g/L tryptone, 5 g/L yeast extract) at 37°C and 200 rpm in baffled flasks or plated on LB-Agar as it is not declared otherwise. When strains harboring plasmid pTrc99A and its derivatives, 100 μg/mL ampicillin was supplemented to the medium. Strain YCC202 and its derivatives were supplemented with 25 μg/ml streptomycin and 10 μg/ml tetracycline, additionally.
Table 4 Strains, plasmids and oligonucleotides used in this study

| Strains | Relevant characteristics | Reference |
|---------|--------------------------|-----------|
| E. coli DH5α | F’ thi-1 endA1 hsdR17 (rK’-m’-) supE44 ΔlacU169 (φ80lacZAM15) recA1 gyrA96 relA1 | [19] |
| E. coli W3110 | F’λ INVmD – mE1 | [19] |
| E. coli MG1655 | F’λ incG- rfb-50 rph-1 | [19] |
| E. coli YCC202 | ΔaceE pφ1 psx81 pps4 ppsL zbi: Tn10 | [8] |
| E. coli BW25113 | lacIqrmB8IαlacZAM15 hsdR514 araBAD Δ(hsdR514)oniaB ΔDE3 | [20] |
| JW0855-1/pTrc99A-ald-adj-ta<sub>Π</sub> (BW25113Δpro88kan) | F<sup>+</sup> ΔaroA-ΔaraB567 ΔlacZ478::rrnB-3), λ<sup>+</sup> ApoxR772::kan, rph-1, Δhoda::hdaB568, hsdR514; harboring the plasmid pTrc99A-ald-adj-ta<sub>Π</sub> with the transaminase of Chromobacterium violaceum | This study |
| JW2293-1/pTrc99A-ald-adj-ta<sub>Π</sub> (BW25113Δark4-kan) | F<sup>+</sup> ΔaroA-ΔaraB567 ΔlacZ478::rrnB-3), λ<sup>+</sup> Δack778::kan, rph-1, Δhoda::hdaB568, hsdR514; harboring the plasmid pTrc99A-ald-adj-ta<sub>Π</sub> with the transaminase of Chromobacterium violaceum | This study |
| JW2294-1/pTrc99A-ald-adj-ta<sub>Π</sub> (BW25113Δprazkan) | F<sup>+</sup> ΔaroA-ΔaraB567 ΔlacZ478::rrnB-3), λ<sup>+</sup> Δpta779::kan, rph-1, Δhoda::hdaB568, hsdR514; harboring the plasmid pTrc99A-ald-adj-ta<sub>Π</sub> with the transaminase of Chromobacterium violaceum | This study |

| Plasmids | Relevant characteristics | Reference |
|----------|--------------------------|-----------|
| pTrc99A-ta<sub>Π</sub> | pTrc99A carrying ta<sub>Π</sub> of Vibrio fluvialis | This study |
| pTrc99A-ta<sub>Π</sub> | pTrc99A carrying ta<sub>Π</sub> of Chromobacterium violaceum | This study |
| pTrc99A-ald-adj-ta<sub>Π</sub> | pTrc99A carrying ald-adj-ta<sub>Π</sub> synthetic operon | [6] |
| pTrc99A-ald-adj-ta<sub>Π</sub>mut<sub>Π</sub> | pTrc99A-ald-adj-ta<sub>Π</sub> with BamH1 cut site upstream of ta<sub>Π</sub> | This study |
| pTrc99A-ald-adj-ta<sub>c</sub> | pTrc99A carrying ald-adj-ta<sub>c</sub> synthetic operon | This study |
| pTrc99A-ald-adj-ta<sub>c</sub>mut<sub>c</sub> | pTrc99A-ald-adj-ta<sub>c</sub> from B. subtilis 168 | [20] |
| pTrc99A-ald-adj-ta<sub>c</sub> | pTrc99A-ald-adj-ta<sub>c</sub> from C. violaceum | |

| Oligonucleotides | Sequence 5’→3’ | Use |
|-----------------|----------------|-----|
| ta<sub>Π</sub>_RBS_for | CAGACCCATGGAAATTCGAGCAGGAAAACACAGCATGAACAAACCACGAGCTG | pTrc99A-ta<sub>Π</sub> |
| ta<sub>Π</sub>_rev | ATCCCGCGGTTTCCGCGTACGCTCGGAAAAC | pTrc99A-ta<sub>Π</sub> |
| ta<sub>c</sub>_KpnI_RBS_for | CAAAGTTCCCCGAAACAGCATGCAGAAACACGCTACCC | pTrc99A-ta<sub>c</sub> |
| ta<sub>c</sub>_BamH1 rev | GTGGATCCCTAGGCAGACCCGCTGCTT | pTrc99A-ta<sub>c</sub> |
| pTrc99A-ald-adj-ta<sub>c</sub>mut<sub>c</sub>_for | GGAATATGATAAGATCCGGATGACTGACATGAACACCGCATGACC | pTrc99A-ald-adj-ta<sub>c</sub> |
| pTrc99A-ald-adj-ta<sub>c</sub>mut<sub>c</sub>_rev | CTGGGATCCCTAGGCAGACCCGCTGCTT | pTrc99A-ald-adj-ta<sub>c</sub> |
| pTrc99A-ald-adj-ta<sub>c</sub>_for | CAAGGATCCCCGAAACAGCATGCAGAAACACGCTACCC | pTrc99A-ald-adj-ta<sub>c</sub> |
| pTrc99A-ald-adj-ta<sub>c</sub>_rev | GTGGATCCCTAGGCAGACCCGCTGCTT | pTrc99A-ald-adj-ta<sub>c</sub> |
Preparation of cell free extract and enzyme assay

The E. coli derivatives were grown in LB + 100 μg/mL ampicillin until an optical density at 600 nm of 0.6 – 0.8, induced with 1 mM isopropyl-β-D-thiogalactopyranosid (IPTG) and harvested in the exponential phase at OD₆₀₀ = 3.5. 10 mL of the cell culture was harvested and always kept on ice. The cells were once washed with buffer for the enzyme assay, resuspended in 1 mL of the same buffer and then lysed by sonication (Ultrasonic processor UP200S, Hielser Ultrasonic Technology, Teltow, Germany) for 2 minutes (cycle 0.5; amplitude 55%). Cell cebris was centrifuged at 10.000 x g at 4°C for 1 hour and clear cell extract was used for the measurement of the enzyme activity.

Measurement of alanine dehydrogenase activity

50 mM Na₂CO₃ pH 10 was used for cell washing. For measuring the activity of alanine dehydrogenase pyruvate was converted to L-alanine by NADH consumption spectrophotometrically at 340 nm. Therefore, 50 mM Na₂CO₃ pH 8.5, 50 mM NH₄Cl, 10 mM pyruvate and 0.25 mM NADH where mixed in a cuvette, filled up to 1 mL ddH₂O and upon the addition of crude extract the reductive amination was initiated and measured for 3 minutes. The assay was performed in triplicates and one enzyme unit was calculated to be the amount of enzyme that catalyzes the conversion of 1 μmol substrate in 1 min.

Measurement of alcohol dehydrogenase activity

25 mM Sodium phosphate buffer pH 8 was used for cell washing. For measuring the alcohol dehydrogenase activity 1,4-butanediol was oxidized to hydroxybutyraldehyde and NADH formation followed spectrophotometrically at 340 nm. Therefore, 25 mM Na-P-buffer pH 8, 18 mM 1,4 butanediol and 10 mM NAD⁺ were mixed in a cuvette, filled up to 1 mL ddH₂O and reaction was initiated upon the addition of crude extract (triplicates). The NADH formation was followed over 3 minutes and one enzyme unit was calculated to be the amount of enzyme that catalyzes the conversion of 1 μmol substrate in 1 min.

Measurement of transaminase activity

100 mM Potassium-phosphate buffer pH 7.4 was used for cell washing. Reaction conditions were: 100 mM K-P-buffer pH 7.4, 50 mM (S)-α-MBA and 10 mM pyruvate. The transamination was initiated upon the addition of crude extract and samples were taken continuously. The reaction was stopped with 75 μL 16% perchloracetic acid. The samples where neutralized by the addition of 40 μL buffer containing 20 mM Tris/HCl pH 8 and 23 mM K₂CO₃, L-alanine formation was measured via HPLC and one enzyme unit was calculated to be the amount of enzyme to catalyze the formation of 1 μmol product in 1 min.

The experimental procedure for the estimation of catalytic efficiency was equal but hexanal was used as substrate instead of (S)-α-MABA. The Reaction conditions were: 100 mM K-P-buffer pH 7.4, 10 mM hexanal and varying concentrations of L-alanine. Helylamine formation was measured via HPLC and one enzyme unit was calculated to be the amount of enzyme to catalyze the formation of 1 μmol product in 1 min.

Whole cell biotransformation with resting cells

E. coli W3110/pTrc99A and its derivatives W3110/pTrc99A-ald-adi-ta and W3110/pTrc99A-ta-ald-adi were inoculated to an initial OD₆₀₀ = 0.1 in LB-medium plus 20 mM Mops and 100 μg/mL ampicillin and incubated at 37°C and 200 rpm. At an OD₆₀₀ = 0.6-0.8 1 mM IPTG was added to the expression culture to induce the cells and cultivation was continued as described above. 15 hours cells were harvested for a final OD₆₀₀ = 10 in 20 mL final volume, once washed with 50 mM Hepes buffer pH 7 and prepared for whole cell biotransformation in a resting buffer system with the mentioned buffer. NH₄Cl and L-alanine were added to the system when necessary and concentrations are given in the text. The test reaction containers (100 mL Schottbottle) where incubated at 37°C or 42°C and 200 rpm and samples for HPLC-analytics were taken in intervals throughout the production.

HPLC-analysis

Extracellular amines and 1-amino-10-decanol were analyzed by high-pressure liquid chromatography (HPLC, 1200 series, Agilent Technologies Deutschland GmbH, Böblingen, Germany). Samples were centrifuged at 10.000 x g for 5 minutes and the clear supernatant was taken for HPLC-measurement. For the detection samples were derivatized with ortho-phthalaldehyde (OPA) automatically before entering the precolumn (LiChrospher 100 RP8 EC-5 μ, 40 × 4.6 mm, CS-Chromatographie Service GmbH, Langerwehe, Germany) and the main column (LiChrospher 100 RP8 EC-5 μ, 125 × 4.6 mm, Langerwehe, Germany) for separation. The used mobile phase was made of A: 0.25% (v/v) Na-acetate buffer pH 6 and B: Methanol; 0 min 30% B, 1 min 30% B, 8 min 70% B, 13 min 90% B, 16 min 70% B, 18 min 30% B. 1,7-diaminoheptane was used as internal standard.

The detection of amino acids were performed with a quicker HPLC-method but derivatization with OPA was used equally to amine detection. Here, through a precolumn (LiChrospher 100 RP 18–5 EC; 40 × 4 mm) and the main column (LiChrospher 100 RP18 EC-5 μ; 125 ×
4.6 mm; CS-Chromatographie Service GmbH, Langerwehe, Germany) amino acids were separated and detected by a FLD-detector. As an internal standard L-asparagine was used and the gradient for improved separation was made of A: 100 mM Sodiumacetate pH 7.2 and B: Methanol; 0 min 25% B, 0.5 min 45% B, 4 min 65% B, 7 min 70% B, 7.2 min 80% B, 7.4 min 85% B, 8 min 20% B, 10.6 min 20% B.

Overflow metabolites were separated by the Organic Acid Resin column (800 × 8 mm) from CS-Chromatographie Service GmbH (Langerwehe, Germany) and detected with DAD-detector. An isotropic elution with 5 mM H2SO4 and a flow rate of 0.7 ml/min for the separation of the samples.

Competing interests

The authors declare that they have no competing interests.

Authors’ contribution

VFW and SK designed the experiments. SK conducted the experiments, analyzed the results and wrote the manuscript. VFW reviewed and revised the manuscript. Both authors read and approved the final manuscript.

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