

Enantioselective C–C Bond Ligation Using Recombinant *Escherichia coli*-Whole-Cell Biocatalysts

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Abstract: Thiamine diphosphate (ThDP)-dependent enzymes like benzaldehyde lyase from *Pseudomonas fluorescens* (BAL) and benzoylformate decarboxylase from *Pseudomonas putida* (BFD) are versatile biocatalysts for the C–C bond ligation of aldehydes to form enantiomerically pure 2-hydroxy ketones. However, the large-scale application of this enzyme class is often restricted by the required external addition of the expensive cofactor ThDP, as well as by the common use of dimethyl sulfoxide (DMSO) as a cosolvent, which leads to problems during the work-up procedure. In the present paper we demonstrate that the addition of the excess cofactors, ThDP and magnesium ions (Mg^{2+}), is not required when BAL or BFD are used in *Escherichia coli* resting cells. Furthermore, the combination of these resting cells with a biphasic reaction medium [methyl *tert*-butyl ether (MTBE)/aqueous buffer] allows an increase of the

substrate concentration up to 1 M, and an efficient extractive work-up. As a practical example, e.g., the synthesis of (*R*)-2-hydroxy-3,3-dimethoxy-phenylpropanone from benzaldehyde and 2,2-dimethoxyacetaldehyde was optimized, achieving an isolated yield of 78 %, and an enantiomeric excess of 98 % *ee* in 24 h when operating at a substrate concentration of 0.4 M. The described reaction system in a biphasic medium is suitable for a wide range of aldehydes as substrates. The biphasic reaction medium minimizes also the formation of by-products, which were observed when this reaction was performed in the conventional DMSO/buffer system.

Keywords: benzaldehyde lyase; C–C bond formation; cofactors; enzyme catalysis; multiphase catalysis; resting cells; thiamine diphosphate

Introduction

Efficient synthetic strategies and industrially feasible processes are key factors for the successful implementation of new chiral drugs. Biocatalysis contributes to this demand due to its inherent advantages like high chemo- and stereoselectivity.^[1] However, for industrial processes also issues related to cost effectiveness have to be addressed. Conclusively, biocatalysts with high activities, as well as high stabilities, leading to high volumetric productivities, are desirable. Moreover, avoiding additional cost factors like the external addition of cofactors are also of high importance for the development of an industrial biocatalytic process.

Optically active 2-hydroxy ketones are important building blocks for the synthesis of several pharmaceuticals and natural products.^[2] Accordingly, some synthetic routes have been developed, ideally avoiding harsh reaction conditions, and/or the use of hazardous reactants.^[3] Over the last years, several representatives of thiamine diphosphate (ThDP)-dependent enzymes have proved to be highly efficient biocatalysts for the carbonylation of aldehydes. Thus, especially aromatic 2-hydroxy ketones like benzoin, phenylacetyl carbinol (PAC), or 2-hydroxypropionophenones (2-HPP), are formed in high yields – and usually in an enantiomerically pure form – with this biocatalytic toolbox.^[4] Depending on the sequence with

which the aldehyde substrates are ligated by the biocatalyst, a donor-acceptor concept has been developed.^[5]

In this area, benzaldehyde lyase from *Pseudomonas fluorescens* biovar I (BAL)^[6] is a versatile enzyme that shows a wide substrate spectrum. Thus, it catalyzes not only the “classical” benzoin condensation, as well as the enantioselective benzoin cleavage, but also the cross-condensation of benzaldehyde and acetaldehyde as well as derivatives like 2,2-dimethoxyacetaldehyde or phenylacetaldehyde.^[4,5] Its crystalline structure was recently reported^[7] and some variants have already been reported as well.^[8] In addition, benzoylformate decarboxylase from *Pseudomonas putida* (BFD) is a second promising ThDP-dependent enzyme useful for the enantioselective ligation of aldehydes to yield 2-hydroxy ketones.^[3,4h,i,j] Also several BFD variants have already been reported.^[9] Moreover, the enantioselectivity displayed by BFD is often complementary to BAL.^[10] The structural reasons for the differences in selectivity have recently been elucidated.^[11] So far both enzymes have been applied in synthetic processes as free enzymes by the addition of ThDP and Mg²⁺ as cofactors to the reaction buffer, in order to keep the holo-enzymes stable. The substrate and product concentrations were usually in the range of 20–50 mM. Recently, some of us showed that the substrate concentration can be increased up to 500 mM for the BAL-catalyzed benzoin condensation, and up to 130 mM for the formation of 2-HPP by adding the substrate in a dosage mode in a buffer/DMSO monophasic reaction medium.^[12] However, from a practical point of view an even higher increase in substrate/product concentration would be beneficial. As the use of DMSO as a cosolvent causes problems during the extractive work-up, the application of biphasic systems has turned out to be an important tool within enzymatic catalysis.^[13] Further the application of immobilized BAL and BFD has been part of several investigations.^[14]

A high stability of the biocatalyst is a further key factor for the technical application of such an enzymatic process. Therefore, immobilization techniques and reactor design are frequently used strategies for an efficient use and a low consumption of the biocatalyst.^[15] As an alternative, the resting cell concept represents an elegant and practical methodology for the efficient application of enzymes,^[16] and becomes more and more attractive as a versatile, economical and highly efficient catalytic methodology in organic synthesis.^[17] For redox reactions the application of resting cells is advantageous, as the regeneration of the expensive redox cofactor is performed within the resting cell.

Here we report the first BAL- and BFD- catalyzed reactions catalyzed by recombinant whole cell catalysts. In contrast to the analogous reactions with

“free” enzymes, addition of cofactor (ThDP) to the reaction buffer is not required. Furthermore, we combined this successful resting cell strategy with a biphasic reaction medium, which leads to an enhancement of the substrate and product concentration and to an effective extractive work-up.

Results and Discussion

BAL in Biphasic Media

The establishment of a biphasic process of BAL-catalyzed carbonylation using whole cells was started with the screening for suitable organic solvents, taking the results from the studies of free BAL in a two-phase system into account.^[18] In a first step the stability of BAL against different classes of solvents was examined.^[19] Eight different organic solvents (heptane, isohexane, cyclohexane, toluene, dichloromethane, diethyl ether, 1-octanol and ethyl acetate) were selected representing the main classes of solvents. In that respect, the corresponding log *P* values were calculated according to the fragmentation method reported by Crippen et al.^[20,21] Therefore, the enzyme was dissolved in potassium phosphate buffer containing Mg²⁺ and ThDP. Once the initial activity of the enzyme in the water phase was measured, the same amount of organic solvent was added. Figure 1 shows the stability of BAL in presence of the organic solvents applied in this solvent screening.

As observed from Figure 1, no correlation between the log *P* value of the solvent and BAL stability can be observed. Aromatic and aliphatic alkanes, as well as esters and halogenated solvents lead to a fast enzyme deactivation. High activity maintenance is achieved with 1-octanol serving as organic phase. Notably, 1-octanol has also been used by other authors with analogous ThDP-dependent enzymes.^[4a,b] Also, a

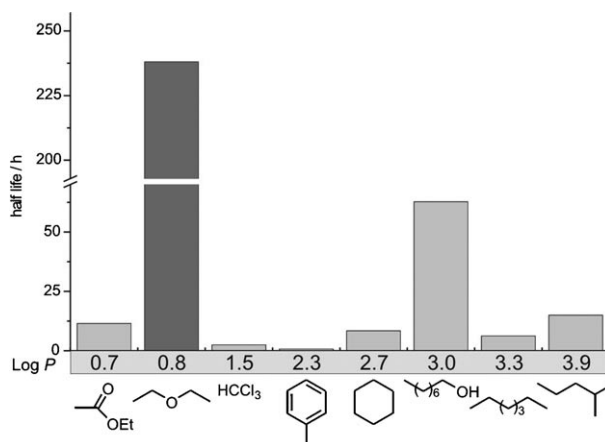


Figure 1. Half life of BAL in aqueous/organic biphasic systems (storage stability).

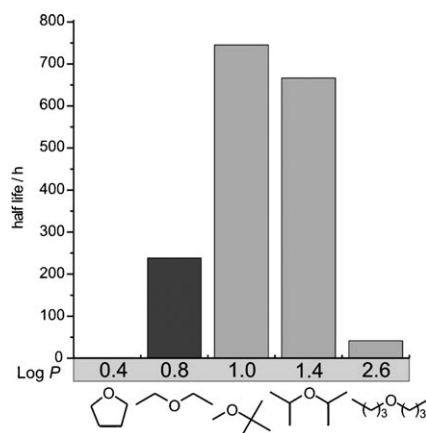


Figure 2. Half life of isolated BAL with different ethers serving as second phase (storage stability).

high activity remains in the presence of diethyl ether. Therefore, in a second step we decided to screen for other ethers. Finally, tetrahydrofuran was tested representing cyclic ethers, diethyl and dibutyl ether as representatives of ethers with non-branched aliphatic chains; *tert*-butyl methyl ether and diisopropyl were chosen representing ethers with bulky alkyl chains.

As shown in Figure 2, BAL displays a high compatibility with many ethers (with the exception of THF and dibutyl ether) compared with the solvents tested in the first screening (Figure 1). In the presence of ethers with branched alkyl chains a remarkable stability ($t_{1/2} > 600$ h) and no inhibition was observed (inhibition data not shown). Once again no correlation between the log P values of the tested ethers and the stability of the biocatalyst was found. The enzyme stability is mainly affected by properties based upon the molecule structure of the tested ethers. Consequently, and also due to the absence of peroxide formation, MTBE was applied in the biotransformation with BAL. Moreover, MTBE provides suitable extractive work-up conditions, fulfils the economic requirements of a solvent,^[18] and has been successfully employed in other enzyme-based processes.^[13]

BAL-Catalysis with Resting Cells in Biphasic Media without Addition of ThDP

The MTBE/aqueous buffer was subsequently used as a reaction media in the development of a whole cell process. As a model reaction the BAL-catalyzed carbonylation of benzaldehyde and dimethoxyacetaldehyde to yield (*R*)-2-hydroxy-3,3-dimethoxyphenylpropanone (**4**) was chosen. Recently, this synthetically valuable reaction was done with crude extract in a

monophasic aqueous medium (buffer/DMSO), at substrate concentrations of 20 mM.^[4d]

This reaction catalyzed by isolated BAL was carried out in a biphasic medium consisting of MTBE and phosphate buffer. Optimal reaction conditions were: 400 mM benzaldehyde and 1.2 M 2,2-dimethoxyacetaldehyde (both concentrations are referred to the organic phase), *ca.* 400 U mL⁻¹ (referred to the aqueous phase) of BAL in MTBE:phosphate buffer system (100 mM; pH 9.5, 2.5 mM MgSO₄; 0.3 mM ThDP). Subsequently, we compared the activity of BAL in resting whole cells versus BAL in a cell crude extract obtained by disrupting one cell fraction of the same cultivation batch by sonification. These experiments were performed in the presence as well as in the absence of added cofactors. The product concentration time course of these four experiments is depicted in Figure 3.

As depicted in Figure 3, the use of crude cell extracts with added cofactors leads to the formation of 345 mM of **4**, which corresponds to a yield of 86% within 24 h (■). In the absence of cofactors the crude extract does not catalyze the carbonylation (◇). Notably, the performance of the system with resting cells is similar to the crude cell extract plus added cofactors and is not influenced by the addition of cofactors to

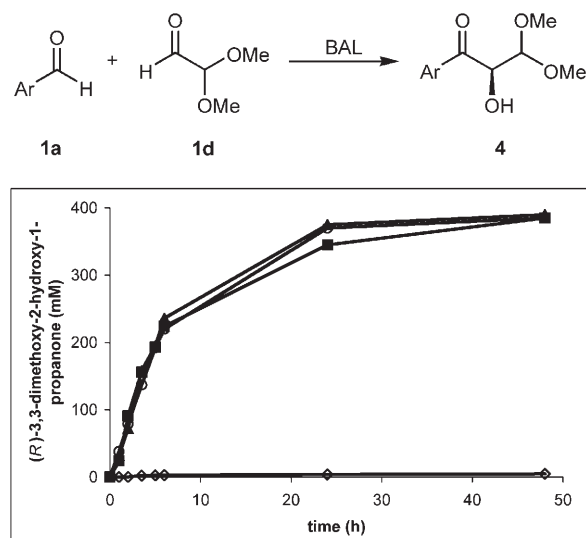


Figure 3. BAL-catalyzed carbonylation of benzaldehyde (**1a**) and 2,2-dimethoxyacetaldehyde (**1d**) yielding (*R*)-3,3-dimethoxy-2-hydroxy-1-phenylpropanone (**4**) with resting cells or free enzyme in biphasic system, with or without added cofactors (◇: crude cell extract without cofactor; ■: crude cell extract with cofactor; ▲: resting cells without cofactor; ○: resting cells with cofactor). Benzaldehyde (**1a**) (400 mM); 2,2-dimethoxyacetaldehyde (**1d**) (1.2 M); MTBE, phosphate buffer (100 mM, pH 9.5); 2.5 mM MgSO₄; 0.3 mM ThDP with/without cofactor; 1 g *E. coli* cells with over-expressed BAL or equivalent crude cell extract produced by 1 g cells (40 g L⁻¹).

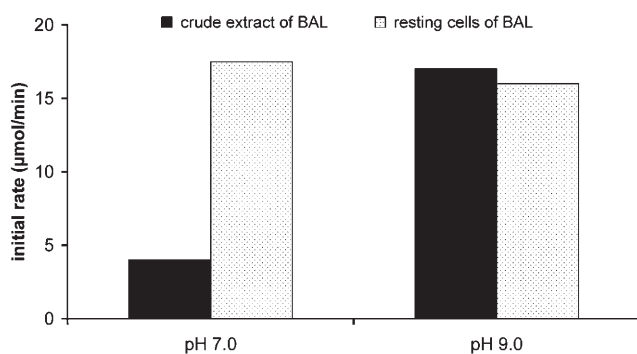


Figure 4. Initial rate for the BAL-catalyzed synthesis of (*R*)-2-hydroxy-3,3-dimethoxyphenylpropanone (**4**) in a biphasic system with crude cell extracts (filled bar) and resting cells (dotted bar) at pH 7.0 and 9.0, respectively. Benzaldehyde (**1a**) (400 mM); 2,2-dimethoxyacetaldehyde (**1d**) (1.2 M); MTBE; phosphate buffer (100 mM, pH 7.0 or 9.5); 2.5 mM MgSO₄; 0.3 mM ThDP; 1 g *E. coli* cells with over-expressed BAL or equivalent crude cell extract produced by 1 g cells (40 g L⁻¹).

the reaction medium (○, ▲). Therefore, neither external ThDP nor Mg²⁺ are necessary for the carbonylation when resting cells are used. To the best of our knowledge, this is the first example of the application of recombinant resting cells for ThDP-dependent enzymes that does not need external addition of costly cofactors.

Recently, we have reported that the pH is a key parameter in the ThDP-dependent enzymes performance of BAL, with an optimum at pH 9.5 in a monophasic aqueous system.^[12] Comparable results were obtained with crude cell extracts (Figure 4). Interestingly, when BAL whole cells were tested, the enzymatic activity was independent of the buffer pH value (range 7.0 to 9.0), and the enzymatic performance was the same in all cases.

Substrate Range of BAL/BFD in Resting Cells in Biphasic Reaction Media

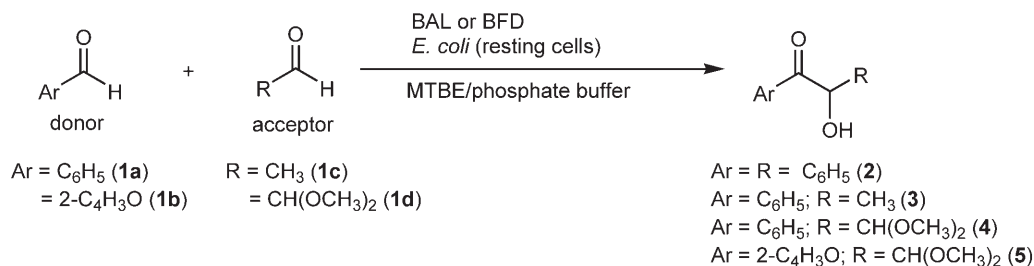
As previously published, BAL has the broadest substrate range compared to other ThDP-dependent en-

zymes catalyzing carbonylation reactions, which makes this enzyme attractive for preparative purpose.^[3,4c-g] In order to compare the performance of the whole cell system with data previously obtained with isolated enzyme, the substrate range of BAL has been investigated with resting cells in a biphasic MTBE/phosphate buffer system. Results are summarized in Scheme 1 and Table 1, including also an example with BFD.

As shown in Table 1 the resting cell concept for BAL has been successfully applied to different aldehydes, most of them known to be substrates for BAL as an isolated enzyme. Thus, both the self-condensation of benzaldehyde to benzoin, as well as the cross-condensation of benzaldehyde with other aldehydes takes place in high yields. In addition, due to the biphasic system it is possible to operate at high substrate concentrations. Compared to reactions with isolated enzyme in monophasic systems with a typical substrate concentration in the range of ca. 20–50 mM, the new reaction conditions allow a 10–20 fold increase in the substrate concentration. Moreover, in most of the cases an overall isolated yield of > 70% – obtained after non-optimized extractive work-up – was achieved. The stereoselectivities remained unchanged relative compared to those reported for the isolated enzymes in monophasic aqueous media.^[4]

Compared to the monophasic system,^[12] it was possible to enhance the maximum concentration of benzaldehyde for the benzoin synthesis by a factor of two to 1 M. Moreover, for the synthesis of 2-HPP (**3**) and 2-hydroxy-3,3-dimethoxy-1-phenylpropanone (**4**) the substrate concentration of benzaldehyde was enhanced from about 20 mM as described^[4] to 1 M of **1c** and to 0.4 mM of **1d**, respectively, whereas the concentration is referred to the volume of the organic phase. In all cases, stereoselectivity remained the same as with isolated enzymes.

Furthermore, the resting cells/biphasic system concept also turned out to be suitable for BFD-catalyzed carbonylation reactions (Table 1, entry 4). Thus, the BFD-catalyzed formation of (*S*)-2-HPP proceeded with a yield of 60% and high *ee* (94%) in 20 h when applying benzaldehyde and acetaldehyde in high concentration (0.3 M). As has been previously publish-



Scheme 1. Carbonylation with *E. coli* cells containing over expressed BAL and BFD respectively with various aldehydes in biphasic system and without external addition of ThDP.

Table 1. Carboligation in biphasic system with recombinant resting *E. coli* cells containing over expressed BAL and BFD.

Entry	Donor	Acceptor	Product	Enzyme	Isolated yield ^[a]	Time	ee (excess enantiomer) ^[b]
1	1a (0.5 M) ^[c]	1a (0.5 M) ^[c]	benzoin (2)	BAL	80 %	24 h	> 99 % (<i>R</i>)
2	1a (0.5 M)	1c (0.65 M)	2-hydroxy-1-phenylpropanone (3) ^[d]	BAL	70 %	24 h	98 % (<i>R</i>)
3	1a (1.0 M)	1c (1.25 M)	2-hydroxy-1-phenylpropanone (3) ^[d]	BAL	71 %	24 h	98 % (<i>R</i>)
4	1a (0.3 M)	1c (0.9 M)	2-hydroxy-1-phenylpropanone (3)	BFD	60 %	20 h	94 % (<i>S</i>)
5	1a (0.4 M)	1d (1.2 M)	3,3-dimethoxy-2-hydroxy-1-phenylpropanone (4)	BAL	78 %	20 h	98 % (<i>R</i>)
6	1b (0.4 M)	1d (1.2 M)	3,3-dimethoxy-2-hydroxy-1-furan-2-yl-propanone (5)	BAL	90 %	16 h	98 % (<i>R</i>)

^[a] 25 mL MTBE, 25 mL phosphate buffer (100 mM, pH 9.5), 40 g/L of *E. coli* cells containing over-expressed BAL or BFD, respectively. No extra addition of ThDP or Mg²⁺.

^[b] Absolute configuration according to previous literature.^[4]

^[c] Total concentration of aldehyde: 1 M. The concentration refers to the volume of the organic phase.

^[d] Dosage mode.

ed,^[4h] the enantioselectivity of BFD for the synthesis of 2-HPP was reported to vary between 88 and 94 % ee, being higher at lower benzaldehyde concentrations. Our recent results are in agreement with those findings, since within a biphasic reaction media with resting cells, most of the benzaldehyde will remain in the organic phase, and thus the effective concentration of the donor aldehyde nearby the enzyme will be low. Thus, the present system is advantageous over the monophasic system with isolated BFD as it does not require an enzyme-membrane reactor in order to maintain low benzaldehyde concentrations throughout the reaction and can be run in a simple batch reactor.^[4] Furthermore, both aldehydes, benzaldehyde and acetaldehyde, can be applied in a concentration ratio of 1:3, whereas the monophasic reaction with isolated BFD required an about 100-fold excess of acetaldehyde.

Optimizing the BAL-Catalyzed Synthesis of 2-HPP in the Biphasic System

During enzymatic carboligation the ratio of both aldehydes is of great importance especially for preparative applications. With respect to purification procedures complete conversion of an equimolar mixture would be desirable. Thus, we tried to minimize the ratio of both aldehydes in the cross-condensation of benzaldehyde and acetaldehyde in the biphasic system. In monophasic reaction media a ratio of acetaldehyde: benzaldehyde of 6:1 was found to be advantageous for the formation of the mixed product 2-HPP.^[12] In our experiments with the biphasic reaction medium we found that a much lower ratio was sufficient to fully assure the 2-HPP synthesis catalyzed by BAL. This might be due to the different partition coefficient

of benzaldehyde and acetaldehyde in the aqueous and organic phase.

In contrast to previous experiments, we also observed the formation of the undesired by-product phenylacetyl carbinol **6** (PAC) which was determined by NMR and GC. The amount of formed PAC depends on the ratio of benzaldehyde and acetaldehyde in the biphasic reaction system. According to Figure 5, a 2-fold surplus of benzaldehyde relative to acetaldehyde is necessary in the biphasic system to achieve a 200-fold excess of 2-HPP over PAC. However, with this approach only 50 % of the benzaldehyde would be available for the production of 2-HPP, and concomitantly to the formation of benzoin as an intermediate, as previously reported.^[4e,12] Notably, in a monophasic aqueous reaction system the formation of PAC was not reported.^[4]

We solved this problem by using a dosage technique with respect to acetaldehyde addition, starting with the initial portion of benzaldehyde and adding dropwise in periodic time intervals 50 mM of acetaldehyde. Using these reaction conditions we isolated 70 % of pure (*R*)-2-HPP with a high enantiomeric excess of 98 % (Table 1, entry 3) and no PAC could be detected now. Moreover, as an additional advantage, we achieve a nearly equimolar ratio of 1.25 between acetaldehyde and benzaldehyde. This is much more favourable than in a monophasic aqueous reaction media, where a 6-fold surplus of acetaldehyde was found to be best.^[12]

Conclusions

In summary, enzymatic carboligation of aldehydes can be performed very efficiently by resting cells in biphasic medium. Hereby, no deactivation of the enzyme was observed. Notably, the addition of external cofac-

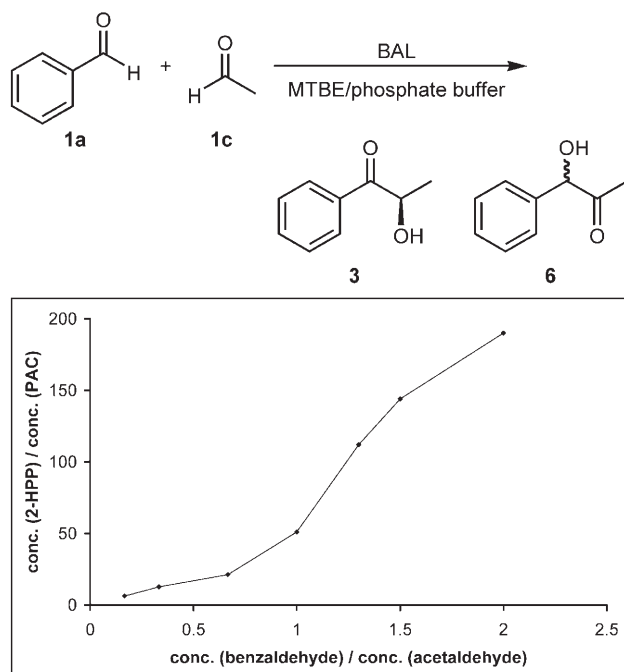


Figure 5. Relationship between 2-HPP/PAC vs. [benzaldehyde]/[acetaldehyde] in the BAL-catalyzed carbonylation. Benzaldehyde (**1a**) (400 mM); variable amounts of acetaldehyde; MTBE; phosphate buffer (100 mM, pH 8); 1 g *E. coli* cells with over-expressed BAL (40 g L⁻¹).

tors (ThDP and Mg²⁺) is not necessary with resting cells containing BAL and BFD. Moreover, the set-up of a biphasic reaction medium allows an increase of substrate concentrations up to 10-fold compared to the monophasic system without a decline in selectivity and facilitates the extractive work-up. For this purpose MTBE was chosen as the best organic solvent both for the isolated enzymes and for whole cells. Several aldehydes have successfully been used as substrates and were ligated with high enantioselectivities and yields in most cases. Finally, the biphasic media is also useful to avoid the formation of some by-products which are usually found in monophasic aqueous systems.

Experimental Section

Chemicals and Biocatalyst

All reagents were commercially available from Sigma-Aldrich and were used without further purification. Benzaldehyde lyase (BAL) from *Pseudomonas fluorescens*, and benzoylformate decarboxylase (BFD) from *Pseudomonas putida* were produced as described elsewhere.^[4b,6a,8a]

In a typical procedure (crude extract), 2 g of *E. coli* cells with over-expressed BAL or BFD (ca. 20% of soluble cell protein) were suspended in 20 mL phosphate buffer (50 mM), with 2.5 mM MgSO₄ and 0.3 mM ThDP, or with-

out addition of cofactors. After cell disruption by sonification (ca. 3–5 min), the solution was centrifuged at 4000 rpm over 20 min at 4 °C. The pellets were removed and the supernatants were used as the crude extract. For reactions using whole cells (mono- or biphasic system), *E. coli* cells with over-expressed BAL or BFD were suspended in the same buffer (without addition of extra cofactor), and used directly as the biocatalysts.

Determination of Protein Concentration and Activity

The protein concentration was determined photometrically using Coomassie brilliant blue (BioRad, Munich) and bovine serum albumin as a standard.^[8a] All activity measurements were performed using standard conditions: 0.06 mmol benzaldehyde were dissolved in 2.9 mL TEA buffer (35 mM, pH 8) containing 0.35 mM ThDP, 0.35 mM MgSO₄ and 30 vol% DMSO. The reaction was started by adding 0.1 mL enzyme solution (0–0.5 µg mL⁻¹ protein content). Aliquots were taken periodically and the amount of the benzoin formed was determined by HPLC. 1 U benzaldehyde lyase was defined as the amount of enzyme which catalyzes the formation of one µmol benzoin per minute starting with 20 mM benzaldehyde at 20 °C. 1 unit of benzoylformate decarboxylase is defined as the amount of enzyme which catalyzes the cleavage of 1 µmol benzoylformate (30 mmol L⁻¹) into benzaldehyde and CO₂ in potassium phosphate buffer, 50 mmol L⁻¹, pH 6.5, containing MgSO₄ (2.5 mmol L⁻¹), ThDP (0.1 mmol L⁻¹) at 30 °C. Quantitative analysis of benzaldehyde and benzoin was performed by HPLC using a LiChrosphere RP-8 column (250 × 4 mm) and TEA buffer (0.2%, pH 3)/acetonitrile (60:40, v:v) as an eluent (flow: 1.0 mL min⁻¹, 20 °C). The enantiomeric excess was determined by chiral phase HPLC with a Daicel Chiralcel OD-H column and 2-propanol/*n*-hexane (98:2, v:v) as an eluent (flow: 1.0 mL min⁻¹, 20 °C).^[4b]

Determination of the Enzyme Stability in the Aqueous/Organic Biphasic System

5 U of purified BAL (obtained according to ref.^[8a]) were dissolved in 2 mL potassium phosphate buffer (50 mM, pH 7.0) containing ThDP (0.5 mmol L⁻¹) and MgSO₄ (0.5 mmol L⁻¹). After addition of the same volume of water-saturated organic solvent, 0.1 mL aliquots were removed from the aqueous phase at intervals and assayed for residual activity. The starting activity was determined prior to solvent addition. To ensure a stable emulsion the solution was stirred with a magnetic stirrer at 700 rpm during the whole course of the experiment ensuring a continuous emulsion and a rapid establishment of the distribution equilibrium. The temperature was kept at 4 °C to avoid thermal deactivation of the enzyme. Samples of the water phase were taken periodically and the remaining activity was measured under standard assay conditions.

The stability of the enzyme in an aqueous/organic biphasic system is influenced by two main factors: i) an abrupt loss of activity (called instantaneous inhibition) immediately after the solvent addition; ii) the enzyme deactivation which leads to a gradual, irreversible loss of activity during course of the experiment. For each solvent the time-dependent loss of activity could be described by a first order exponential decrease [Eq. (1)].

$$A = A_0 \cdot I \cdot e^{-k_{\text{dea}} \cdot t} \quad (1)$$

To consider the instantaneous inhibition, a constant (I) has been introduced, disregarding the type of inhibition (competitive, uncompetitive or non-competitive). The deactivation constant was determined by non-linear curve fitting of Eq. (1) to the time-dependent course of activities for each solvent. The activities were given as relative values relating to the initial activity. The half life ($t_{1/2}$) of the enzyme activity was calculated from the resulting deactivation constant (k_{dea}).

General Procedure for the Carboligation with Resting Cells in a Biphasic System

Aldehydes (substrates) in variable concentrations were dissolved in 25 mL MTBE. Several amounts of *E. coli* resting cells with overexpressed BAL or BFD (0.5–1.5 g, that is, 20–60 g/L reaction) were added to 25 mL phosphate buffer (pH 8.0) without extra addition of cofactor. Both phases were mixed and vigorously stirred for 20–24 h at room temperature. For the work-up, the reaction mixture was extracted with four aliquots of dichloromethane (4 × 50 mL). The organic fractions were collected, dried with MgSO_4 and removed over reduced pressure, yielding different 2-hydroxy ketones. Under such conditions, practically no emulsion was observed during the extraction. The biocatalytic characterization was carried out as described previously.^[4b]

Dosage Mode for the Preparation of 2-Hydroxy-1-phenylpropanone [(R)-3]

Benzaldehyde (2.65 g, 25 mmol) was dissolved in 25 mL MTBE. 1 g *E. coli* resting cells with over-expressed BAL or BFD were added to 25 mL phosphate buffer (pH 8.0) without extra addition of cofactor. Both phases were mixed and vigorously stirred at room temperature. After 30 min the first dose of acetaldehyde (60 mg, 1.25 mmol) was added followed by the next ones with a time-lag of 15 min. After 12 additions the time interval for the doses was set to 30 min and the addition was repeated up to the desired end concentration. The reactions were complete after 24 h.

Analytical Procedure

The reactions and the enantiomeric excesses were followed by chiral GC, employing a Chirasil-DEX CB (Varian), 25 m × 0.32 mm, with an FID detector. The initial temperature (95 °C) was kept constant for 4 min and then rapidly increased (40 °C min⁻¹) to 135 °C. Then a slow slope (1 °C min⁻¹) was set till 160 °C, to assure the enantiomeric separation. Proton and carbon nuclear magnetic resonance (¹H NMR and ¹³C NMR) spectra were determined in CDCl_3 using a Bruker DPX-400.

(R)-Benzoin [(R)-2]:

Following the general procedure 0.5 g *E. coli* cells with over-expressed BAL were added to a solution of 1 M benzaldehyde (**1a**) (25 mL buffer/25 mL MTBE) and stirred at room temperature for 24 h. A white powder was isolated; yield: 2.12 g (80 %, 0.01 mol), >99 % ee; ¹H NMR (CDCl_3): δ =

4.52 (1H, br, OH), 5.95 (1H, s, CH-O), 7.22–7.34 (5H, m, Ar-H), 7.37 (2H, m, Ar-H), 7.50 (1H, m, Ar-H), 7.91 (2H, m, Ar-H).

(R)-2-Hydroxy-1-propionophenone [(R)-3]

Following the general procedure 0.5 g *E. coli* resting cells with over-expressed BAL were added to a solution of 0.5 M benzaldehyde (**1a**) and 0.65 M acetaldehyde (25 mL buffer/25 mL MTBE), and stirred at room temperature for 24 h. White crystals were isolated; yield: 1.31 g (70 %, 8.7 mmol), 98 % ee; ¹H NMR (CDCl_3): δ = 1.45 (3H, d, J = 7.3 Hz, CH_3), 3.80 (1H, s(broad), OH), 5.16 (1H, q, J = 7.3 Hz, CH-O), 7.50 (2H, m Ar-H), 7.62 (1H, m Ar-H), 7.93 (2H, m Ar-H).

(S)-2-Hydroxy-1-propionophenone [(S)-3]

Following the general procedure 0.5 g *E. coli* cells with over-expressed BFD were added to a solution of 0.3 M benzaldehyde (**1a**) and 0.9 M acetaldehyde (25 mL buffer/25 mL MTBE) and stirred at room temperature for 20 h. White crystals were isolated; yield: 1.12 g (60 %, 7.4 mmol), 94 % ee; ¹H NMR (CDCl_3): δ = 1.45 (3H, d, J = 7.3 Hz, CH_3), 3.80 (1H, s br, OH), 5.16 (1H, q, J = 7.3 Hz, CH-O), 7.50 (2H, t, J = 7.5 Hz, Ar-H), 7.62 (1H, tt, J = 7.5 Hz, J = 1.3 Hz, Ar-H), 7.93 (2H, dd, J = 7.5 Hz, J = 1.3 Hz, Ar-H).

(R)-2-Hydroxy-3,3-dimethoxy-1-phenylpropanone [(R)-4]

Following the general procedure 0.5 g *E. coli* cells with over-expressed BAL were added to a solution of 0.4 M benzaldehyde (**1a**) and 1.2 M 2,2-dimethoxyacetaldehyde (25 mL buffer/25 mL MTBE), and stirred at room temperature for 24 h. A viscous oil was isolated; yield: 1.64 g (78 %, 7.8 mmol), >98 % ee; ¹H NMR (CDCl_3): δ = 3.38 (3H, s, CH_3O), 3.42 (3H, s, CH_3O), 4.49 (1H, d, J = 3.6 Hz, OCHO), 5.13 (1H, d, J = 3.6 Hz, CH-O), 7.47 (2H, m, Ar-H), 7.59 (1H, m, Ar-H), 7.98 (2H, m, Ar-H).

(R)-2-Hydroxy-3,3-dimethoxy-1-furylpropanone [(R)-5]

Following the general procedure 0.5 g *E. coli* resting cells with over-expressed BAL were added to a solution of 0.4 M furfuraldehyde and 1.2 M 2,2-dimethoxyacetaldehyde (25 mL buffer/25 mL MTBE) and stirred at room temperature for 24 h. A viscous oil was isolated; yield: 1.89 g (90 %, 9 mmol), >98 % ee; ¹H NMR (CDCl_3): δ = 3.44 (3H, s, CH_3O), 3.46 (3H, s, CH_3O), 4.56 (1H, d, J = 4.1 Hz, OCHO), 4.87 (1H, dd, J = 7.3 Hz, J = 3.9 Hz, CH-O), 6.59 (1H, m, Ar-H), 7.39 (1H, m, Ar-H), 7.67 (1H, m, Ar-H).

Phenylacetyl carbinol (6)

The formation of PAC was determined by ¹H NMR, no isolation was undertaken. ¹H NMR (CDCl_3): δ = 2.04 (3H, s, CH_3), 4.25 (1H, br, OH), 5.09 (1H, s, CH), 7.30 (5H, m, Ar-H),

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