

Review

Biocatalytic reduction of carboxylic acids

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An increasing demand for non-petroleum-based products is envisaged in the near future. Carboxylic acids such as citric acid, succinic acid, fatty acids, and many others are available in abundance from renewable resources and they could serve as economic precursors for bio-based products such as polymers, aldehyde building blocks, and alcohols. However, we are confronted with the problem that carboxylic acid reduction requires a high level of energy for activation due to the carboxylate's thermodynamic stability. Catalytic processes are scarce and often their chemoselectivity is insufficient. This review points at bio-alternatives: currently known enzyme classes and organisms that catalyze the reduction of carboxylic acids are summarized. Two totally distinct biocatalyst lines have evolved to catalyze the same reaction: aldehyde oxidoreductases from anaerobic bacteria and archaea, and carboxylate reductases from aerobic sources such as bacteria, fungi, and plants. The majority of these enzymes remain to be identified and isolated from their natural background in order to evaluate their potential as industrial biocatalysts.

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1 Introduction

Carboxylic acids are available in great abundance from renewable sources, e.g. acetic acid, citric acid, succinic acid, ferulic acid, and many others. Succinic acid, for example, can serve as precursor for the production of bio-based polymers and industry is currently implementing new plants for its production [1]. Also synthetic fatty acids are interesting from the industrial point of view because they can serve as a source for fatty alcohol production, which – in turn – can be used as biofuels, components for the production of detergents, surfactants, and polymers [2]. Increasingly cheaper carboxylic acid production, especially from renewable sources [3], makes them attractive substrates for a whole range of alternative products.

The ultimate goal should be to supplement traditional mineral oil-based intermediates with sustainable technologies. However, to access aldehydes and alcohols from carboxylic acids, efficient reductive processes will become necessary. Industry is met with the problem that carboxylic acid reduction demands strong reduction power.

The carbon atom in the COOH moiety is present at its highest possible oxidation state apart from carbon dioxide and tetrahalogenated carbons. From the thermodynamic viewpoint, this functional group is in an energetically favored state. Consequently, it shows little reactivity and needs a high level of energy for activation in order to participate in chemical reactions. Once this energetic barrier is overcome, the reduction formally yields the respective aldehyde in the first step, which is more reactive than the carboxylic acid (Fig. 1A). Therefore, the aldehyde does not accumulate but is reduced further to the respective primary alcohol. In case the desired product is the aldehyde, appropriate measures need to be taken that prevent this over-reduction. A number of examples for chemical reductions of carboxylic acids to the corresponding aldehydes were reported in the literature (Fig. 1B) [4–6]. However, the routinely used strategy is based on the overreduction to the alcohol and subsequent selective re-oxidation to the aldehyde with the aid of pyridinium

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Abbreviations: **AOR**, aldehyde ferredoxin oxidoreductase; **CAR**, carboxylic acid reductase; **CAV**, 1,1'-carbamoyl methyl-viologen; **MV**, methyl viologen; **PPTase**, phosphopantetheinetransferase; **RTP**, red-colored tungsten-containing protein; **TMV**, 1,1',2,2'-tetramethylviologen

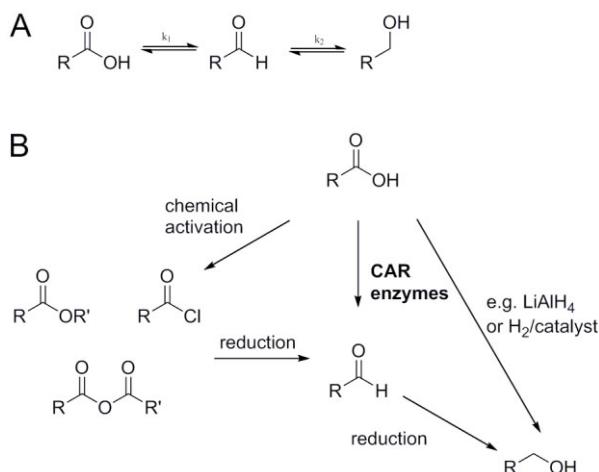


Figure 1. (A) Reduction of carboxylic acids ($k_1 \ll k_2$), k_1 and k_2 – reaction coefficients for the reduction of carboxylic acid to aldehyde (k_1) and aldehyde to alcohol (k_2). (B) A simplified overview of strategies for the reduction of carboxylic acids. The two most often applied routes of carboxylic acid reduction versus the biocatalytic route reviewed herein.

chlorochromate, Dess-Martin periodinane, TEMPO or via Swern oxidation [7]. Most of these methods bear in common that they are either expensive or use environmentally harmful reagents.

The vast majority of chemical reductions of carboxylic acids proceed via the formation of activated derivatives. These may, e.g., be esters or amides, acyl halogenides, anhydrides, or carbonates. Typically, complex metal hydrides such as lithium aluminum hydride and sodium borohydride or variants thereof are used in stoichiometric amounts [8, 9]. Catalytic hydrogenations are frequently performed by heterogeneous metal catalysts, however, also homogenous catalytic hydrogenations are strongly emerging [10]. In the past two decades, several selective chemical reductions to alcohols or aldehydes using hydrosilanes have been described [11].

Today, producers of pharmaceuticals and fine chemicals appreciate enzymatic reactions in general, and also biocatalytic reduction processes are gaining importance [12]. In this review, we provide a comprehensive overview of biocatalytic reductions of carboxylic acids.

Enzyme-catalyzed carboxylic acid/carboxylate reduction is a relatively young field in comparison to the extensively studied and applied enzymatic carbonyl reduction. Like the latter, it offers the typical advantages of bio transformation processes:

- Mild conditions: it proceeds in an aqueous environment at ambient conditions (room temperature, atmospheric pressure);
- Chemoselectivity: other reducible moieties remain unaffected;
- Enantioselectivity: enzymes distinguish enantiomers.

The following chapters summarize the reduction of carboxylic acids by the application of biocatalysts. In most

cases, the applied catalyst consisted of cells rather than isolated enzymes and more often than not, the acids were converted to mixtures of aldehyde and alcohol, assumingly due to background reactions mediated by the respective environment of the enzyme.

To give an idea, which compounds may be converted enzymatically, Tables 1–5 provide an overview of reported substrates. Note that several publications also include lists of non-substrates, which were omitted in Tables 1–5 for clarity.

2 Aerobic carboxylate reductions by fungi

To our knowledge, the first report of a biocatalyzed carboxylate reduction appeared in 1959, in which the white-rot fungus of the division Basidiomycota (*Trametes versicolor*, also known as *Polystictus versicolor*, *Polyporus versicolor*, *Coriolus versicolor*, and several other names) reduced a small number of aromatic carboxylic acids (Table 1, entries: 1, 20–23, 27) as well as 2-naphthoic acid (Table 4, entry 2) to the respective aldehydes and further to the alcohols [13, 14]. Further examples of chloro- and nitrobenzoic acid reduction by *T. versicolor* IFO 4937 as well as aminobenzoic, nitrophenylacetic and chlorocinnamic acid by *T. versicolor* IFO 4941 to the respective alcohols followed [15]. Another species from the genus *Trametes*, namely *T. hirsutus* IFO 4917 reduced 3,4,5-trimethoxybenzoic, 3,4-dimethoxycinnamic and 2-methoxyphenylacetic acid to the respective alcohols [15]. *Psilocybe zapotekorum* DSM 1891 reduced ferulic acid to coniferyl alcohol. Interestingly, no reduction activity towards benzoic and phenylacetic acid and its hydroxyl derivatives was found in this study [15], in contrast to reports by Farmer et al. [13] and Nishida and Fukuzumi [16]. This may be an indication of significant differences of carboxylate reductase expression within one species. In addition to the reduction of ferulic acid to the corresponding aldehyde [17] and alcohol [16], also vanillic acid, vanillin and vanillyl alcohol were observed as metabolites in *Trametes* sp. and *Fomes fomentarius* [17]. This observation gave rise to detailed investigations in several research groups with the aim to generate effective microbial vanillin producers. Similar ferulic acid metabolism was reported in other fungi such as *Sporotrichum pulverulentum* ATCC 32629 [18] and *Pycnoporus cinnabarinus* I-937 [19]. This latter species has been in the limelight of this group and in further experiments they combined two filamentous fungi: *Aspergillus niger* I-1472 was used in a process for the transformation of ferulic acid to vanillic acid, which, in turn, was reduced to vanillin by *P. cinnabarinus* MUCL 39532 [20, 21]. Several aspects of this strategy towards vanillin production were subsequently studied [22–24] and applied [25, 26]. Furthermore, also *Phanerochaete chrysosporium* (another member of Polyporales) was used as a reducing agent both in two

Table 1. Benzoic acid derivatives reported as substrates for carboxylate reductases

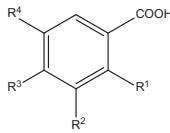
						
	R¹	R²	R³	R⁴	Biocatalyst	Refs.
1	H	H	H	H	<i>Trametes versicolor</i> mycelium <i>Aspergillus niger</i> Perlman Wisconsin 72-4 mycelium <i>Nocardia asteroides</i> JCM 3016, <i>Rhizopus oryzae</i> IFO 5440 and IFO 4706, <i>Mucor fragilis</i> IFO 6449 and <i>Mucor javanicus</i> IFO 4569 and <i>Homoconis resinae</i> F328 resting cells <i>Neurospora crassa</i> SY7A purified CAR ^{a)} <i>Nocardia iowensis</i> sp. NRRL 5646 resting cells, crude extract, purified CAR from it and from heterologous overexpression in <i>E. coli</i> ^{a)} ; also <i>E. coli</i> resting cells heterologously overexpressing NiCAR <i>Clostridium thermoaceticum</i> resting cells ^{b)} Partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^{c)} <i>Clostridium formicoaceticum</i> DSM 92 crude extract ^{d)} Purified W-AOR from <i>Clostridium formicoaceticum</i> DSM 92 ^{e)} <i>Actinomyces</i> sp. ^{f)} <i>Pyrococcus furiosus</i> DSM 3638, growing or resting cells ^{g)}	[13] [34] [35, 36] [45] [46–48, 50] [55] [59] [56] [57] [63] [72, 73]
2	Cl	H	H	H	<i>Trametes versicolor</i> IFO 4937 mycelium <i>Nocardia asteroides</i> JCM 3102 resting cells <i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[15] [35, 36] [46]
3	H	Cl	H	H	<i>Nocardia asteroides</i> JCM 3102 resting cells Partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^{c)} <i>Desulfomicrobium escambiense</i> growing cells <i>Desulfovibrio vulgaris</i> PY1 growing cells and cell free extracts supplemented with electron carrier and donor Purified CAR from <i>Clostridium formicoaceticum</i> DSM 92 ^{e)}	[35, 36] [59] [64] [65] [57]
4	H	Cl	OMe	H	<i>Bjerkandera</i> sp. strain BOS55 mycelium	[31]
5	Cl	H	Cl	H	<i>Desulfovibrio vulgaris</i> PY1 growing cells	[65]
6	Cl	H	H	Cl	<i>Desulfovibrio vulgaris</i> PY1 growing cells	[65]
7	H	Cl	Cl	H	<i>Bjerkandera</i> sp. strain BOS55 mycelium <i>Desulfovibrio vulgaris</i> PY1 growing cells	[31] [65]
8	H	Cl	H	Cl	<i>Desulfovibrio vulgaris</i> PY1 growing cells	[65]
9	H	Cl	OMe	Cl	<i>Bjerkandera</i> sp. strain BOS55 mycelium	[31]
10	H	H	Cl	H	<i>Nocardia asteroides</i> JCM 3102 resting cells <i>Clostridium thermoaceticum</i> resting cells ^{b)} and partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^{c)} Purified CAR from <i>Clostridium formicoaceticum</i> DSM 92 ^{e)} <i>Desulfovibrio vulgaris</i> PY1 growing cells	[35, 36] [59] [57] [65]
11	Br	H	H	H	<i>Nocardia asteroides</i> JCM 3102 resting cells <i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[35, 36] [46]
12	H	Br	H	H	<i>Nocardia asteroides</i> JCM 3102 resting cells <i>Desulfomicrobium escambiense</i> growing cells <i>Desulfovibrio vulgaris</i> PY1 growing cells	[35, 36] [64] [65]
13	H	H	Br	H	<i>Nocardia asteroides</i> JCM 3102 resting cells <i>Desulfovibrio vulgaris</i> PY1 growing cells	[35, 36] [65]
14	H	I	H	H	<i>Nocardia asteroides</i> JCM 3016 resting cells <i>Desulfovibrio vulgaris</i> PY1 growing cells	[35, 36] [65]
15	H	H	I	H	<i>Nocardia asteroides</i> JCM 3016 resting cells	[35, 36]
16	F	H	H	H	<i>Bjerkandera adusta</i> growing cells <i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[30] [46]
17	F	H	H	Me	<i>Bjerkandera adusta</i> growing cells	[30]
18	H	F	H	H	Partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^{c)} Purified W-AOR and Mo-AOR from <i>Clostridium formicoaceticum</i> DSM 92 ^{c)}	[59] [57, 60]

Table 1. Benzoic acid derivatives reported as substrates for carboxylate reductases (continued)

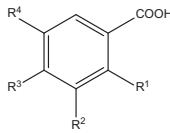
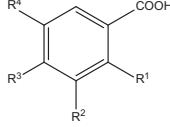
		R¹	R²	R³	R⁴	Biocatalyst	Refs.
19		H	H	F	H	<i>Bjerkandera adusta</i> growing cells	[30]
						<i>Nocardia asteroides</i> JCM 3102 resting cells	[35, 36]
						<i>Desulfovibrio vulgaris</i> PY1 growing cells	[65]
						<i>Bjerkandera adusta</i> growing cells	[30]
						<i>Bjerkandera</i> sp. strain BOS55 mycelium	[31]
						<i>Nocardia asteroides</i> JCM 3016 resting cells	[35, 36]
						<i>Desulfovibrio vulgaris</i> PY1 growing cells	[65]
						Purified CAR from <i>Clostridium formicoaceticum</i> DSM 92 ^e	[57]
20	OMe	H	H	H	H	<i>Clostridium thermoaceticum</i> resting cells DSM 521 ^b and partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^c	[59]
						<i>Trametes versicolor</i> mycelium	[13]
21	OMe	H	OMe	H	H	<i>Neurospora crassa</i> SY7A purified CAR ^a	[45]
						<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^a	[46]
						<i>Neurospora crassa</i> SY7A purified CAR ^a	[45]
22	H	OMe	H	H	H	<i>Trametes versicolor</i> mycelium	[13, 14]
						<i>Aspergillus niger</i> ATCC 9142 and <i>Corynespora cassicola</i> IFO 7483 mycelium	[15]
23		H	OMe	OMe	H	<i>Neurospora crassa</i> SY7A purified CAR ^a	[45]
						<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^a	[46]
						Purified W-AOR and Mo-AOR from <i>Clostridium formicoaceticum</i> DSM 92 ^e	[57, 60]
						Partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^c	[59]
						<i>Trametes versicolor</i> mycelium	[13]
						<i>Bjerkandera</i> sp. strain BOS55 mycelium	[31]
						<i>Actinomyces</i> sp. ^f	[63]
						<i>Trametes</i> sp. mycelium	[16]
24		H	OMe	OH	H	<i>Sporotrichum pulverulentum</i> ATCC 32629 mycelium	[18]
						<i>Pycnoporus cinnabarinus</i> I-937, MUCL 39532 and SW-0204 mycelium	[19–26]
						<i>Phanerochaete chrysosporium</i> ATCC 24725 and MIC 247 mycelium	[27–29]
						<i>Neurospora crassa</i> SY7A purified CAR ^a	[45]
						<i>Nocardia iowensis</i> sp. NRRL 5646 resting cells, crude extract, ^a purified CAR isolated from it and from heterologous overexpression in <i>E. coli</i> ^a	[42, 47, 50]
						<i>Schizosaccharomyces pombe</i> and <i>Saccharomyces cerevisiae</i> growing cells	[51]
						<i>Actinomyces</i> sp. ^f	[63]
						<i>Pyrococcus furiosus</i> DSM 3638 growing cells	[72]
25	H	OMe	OH	OMe	H	<i>Neurospora crassa</i> SY7A purified CAR ^a	[45]
						<i>Actinomyces</i> sp. ^f	[63]
26	H	OMe	OMe	OMe	H	<i>Trametes hirsutus</i> IFO 4917 mycelium	[15]
						<i>Actinomyces</i> sp. ^f	[63]
27		H	H	OMe	H	<i>Trametes versicolor</i> mycelium	[13]
						<i>Corynespora cassicola</i> IFO 7483 mycelium	[15]
						<i>Bjerkandera</i> sp. strain BOS55, <i>Dichomitus squalens</i> CBS 432.34,	[31]
						<i>Phlebia tremellosa</i> ATCC 60027, <i>Phlebia brevispora</i> KBT 89,	
						<i>Phanerochaete chrysosporium</i> ATCC 24725, <i>Schizophyllum commune</i> PW 94.3, <i>Trametes hirsuta</i> CBS 282.73, <i>Trametes versicolor</i> 290,	
						<i>Trametes gibbosa</i> RHEN 93.2, <i>Pleurotus eryngii</i> CBS 613.91,	
						<i>Stereum hirsutum</i> PW 93.4, <i>Lentinus tigrinus</i> PN 94.2 and <i>Polyporus ciliatus</i> ONO 94.1 mycelium	
						<i>Neurospora crassa</i> SY7A purified CAR ^a	[45]
						<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^a	[46]
						<i>Actinomyces</i> sp. ^f	[63]
						Purified AOR from <i>Clostridium formicoaceticum</i> DSM 92 ^e	[57]
						<i>Clostridium thermoaceticum</i> resting cells DSM 521 ^b and partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^c	[59]

Table 1. Benzoic acid derivatives reported as substrates for carboxylate reductases (continued)

						
	R ¹	R ²	R ³	R ⁴	Biocatalyst	Refs.
28	OH	H	H	H	<i>Neurospora crassa</i> cell free extract and purified CAR ^{a)} <i>Aspergillus niger</i> Perlman Wisconsin 72-4 mycelium	[33, 45] [34]
29	H	OH	H	H	<i>Nocardia asteroides</i> JCM 3016 resting cells <i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)} Purified W-AOR from <i>Clostridium formicoaceticum</i> DSM 92 ^{e)} Partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^{c)}	[35, 36] [46] [57] [59]
30	H	OH	OH	H	<i>Neurospora crassa</i> SY7A purified CAR ^{a)}	[45]
31	H	H	OH	H	<i>Aspergillus niger</i> mycelium <i>Nocardia asteroides</i> JCM 3016 resting cells <i>Fomes fomentarius</i> mycelium <i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)} Purified W-AOR from <i>Clostridium formicoaceticum</i> DSM 92 ^{e)} <i>Clostridium thermoaceticum</i> resting cells DSM 521 ^{b)} and partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^{c)}	[34] [35, 36] [17] [46] [57] [59]
32	H	H	OPh	H	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[46]
33	NO ₂	H	Cl	H	<i>Hebeloma sacchariolens</i> fruit bodies and homogenate	[32]
34	H	NO ₂	H	H	<i>Trametes versicolor</i> IFO 4937 mycelium <i>Bjerkandera</i> sp. strain BOS55 mycelium	[15] [31]
35	H	H	NO ₂	H	<i>Trametes versicolor</i> IFO 4937 mycelium	[15]
36	NH ₂	H	H	H	<i>Hebeloma sacchariolens</i> fruit bodies <i>Aspergillus niger</i> Perlman Wisconsin 72-4 mycelium	[32] [34]
37	NH ₂	H	F	H	<i>Hebeloma sacchariolens</i> fruit bodies and homogenate	[32]
38	NH ₂	H	H	F	<i>Hebeloma sacchariolens</i> fruit bodies and homogenate	[32]
39	H	NH ₂	H	H	<i>Trametes versicolor</i> IFO 4941 mycelium <i>Desulfovibrio vulgaris</i> PY1 growing cells	[15] [65]
40	H	NH ₂	OH	H	<i>Streptomyces griseus</i> GriC/GriD purified	[83]
41	H	H	NH ₂	H	<i>Aspergillus niger</i> Perlman Wisconsin 72-4 mycelium	[34]
42	H	H	AcNH	H	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract	[46]
43	Me	H	H	H	<i>Nocardia asteroides</i> JCM 3016 resting cells <i>Nocardia iowensis</i> sp. NRRL 5646 crude extract	[35, 36] [46]
44	H	Me	H	H	<i>Nocardia asteroides</i> JCM 3016 resting cells Purified W-AOR from <i>Clostridium formicoaceticum</i> DSM 92 ^{e)} Partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^{c)}	[35, 36] [57] [59]
45	H	H	Me	H	<i>Nocardia asteroides</i> JCM 3016 resting cells Purified AOR from <i>Clostridium formicoaceticum</i> DSM 92 ^{e)} <i>Clostridium thermoaceticum</i> resting cells DSM 521 ^{b)} and partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^{c)}	[35, 36] [57] [59]
46	Ac	H	H	H	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[46]
47	H	H	Ac	H	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[46]
48	Ph	H	H	H	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[46]
49	H	H	Ph	H	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[46]
50	COOH	H	H	H	<i>Neurospora crassa</i> SY7A purified CAR ^{a)}	[45]
51	H	H	CH ₂ OH	H	Partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^{c)}	[59]
52	H	H	COOH	H	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{b)} and partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^{c)}	[59]

a) In the presence of ATP and NADPH

b) In the presence of CO and MV

c) In the presence of CAV⁺⁺

d) In the presence of CO

e) In the presence of TMV⁺⁺;

f) Lyophilized and permeabilized whole cells

g) In the presence of H₂

step [27, 28] as well as in a one-step vanillin production procedure [29].

The reduction of benzoic acid derivatives was also reported from other Basidiomycota, especially from the genus *Bjerkandera*. All investigated fluorinated benzoic acid substrates (Table 1, entries: 16–19) were metabolized by *Bjerkandera adusta* to the corresponding aldehydes. Interestingly, only the 2-fluorinated derivative was further reduced to the corresponding alcohol [30]. Hage et al. studied the tolerance of different fungi for high concentrations of aryl acids (10 mM) and screened them for the reduction of 4-anisic acid. In course of these studies, 13 fungal strains from different genera were identified with this activity, and typically, a product mixture of aldehyde and alcohol was found. *Phlebia brevispora* KBT 89, by contrast, fully reduced all aldehyde to 4-anisyl alcohol as a product (Table 1., entry 27). *Bjerkandera* sp. strain BOS55 not only reduced 4-anisic acid but also a number of other substrates such as halogenated benzoic acid derivatives (Table 1., entries: 4,7,9,19) [31]. Amine containing benzoic acid derivatives (anthranilic acid and related compounds including *N*-heterocyclic structures) were also subjected to Basidiomycota metabolism and *Hebeloma sacchariolens* was identified as a biocatalyst for reductive transformations of this compound class [32].

Soon after the very first report [13], carboxylate reducing activity has also been found in other fungi from the Ascomycota and Zygomycota. Bachmann et al. reported in vivo reduction of salicylic acid to salicyl alcohol by *Neurospora crassa* [33]. Similarly to the activities found in *H. sacchariolens* [32], mycelium from *A. niger* Perlman Wisconsin 72-4 was reported to convert benzoic acid as well as 2- and 4-aminobenzoic acid to the respective aldehydes [34]. Under anaerobic conditions, benzaldehyde was reduced further [34], similarly to other Aspergilli, which converted the acid substrates to the alcohols. Also resting cells of *Hormoconis resinae* F328 (*Amorphotheca resinae*, fungus from the division Ascomycota) reduced benzoates to the alcohols [35, 36]. Mycelium of *A. niger* ATCC 9142, e.g. acted on 3-methoxybenzoic and 3,5-dimethoxycinnamic acid, and *A. flavus* DSM 1959 reduced 2-methoxycinnamic acid, but only to a very small extent (1.6%). Also *Corynespora cassiicola* (melonis) IFO 7483 was reported to reduce 3-methoxybenzoic, 4-anisic, and also cinnamic acid to the respective alcohols, whereas *C. cassiicola* CBS 12925 only reduced the 2-hydroxy derivative [15]. More recently, also *Mucor* sp. JX23 (division Zygomycota) was shown to reduce cinnamic acid to cinnamic alcohol (Table 2) [37]. *Glomerella cingulata* (the so-called *Malus* strain, because it had been isolated from apples) and *Gloeosporium olivarum* were able to reduce a number of racemic 2-alkyl-2-aryloxyacetic acids to the respective alcohols (Table 5, entry 48) [38, 39]. Notably, the authors observed a kinetic resolution to (*S*) alcohols and (*R*) acids, which is, to our knowledge, the first report of enantioselective enzymatic carboxylate reduction [39].

In a following study, these results were confirmed with further examples in which *G. cingulata* (*Prunus* strain), *G. olivarum* and *Gloeosporium laeticolor* reduced 2-phenyl-, 2-benzylxy- and 2-(2-furfuryl)propionic acid [40]. Finally, *Botrytis cinerea* (four strains: 5889/4, 5901/2, 5882/1, and 5909/1) was the first fungus to be used for the conversion of purely aliphatic substrates (*trans*-3-hexenoic acid and *trans*-3-octenoic acid, Table 5, entries: 25 and 30) to the corresponding alcohols [41].

3 Aerobic carboxylate reductions by bacteria

Mycobacterium phlei DSM 43286 reduced ferulic acid both to aldehyde and alcohol. Interestingly, this species was discovered in the course of a broad screening approach in which diverse species were tested. In that case, more aldehyde (13.1%) than alcohol was found. This indicates that the internal alcohol dehydrogenase(s) are not as active as, for example, in *P. zapotekorum* DSM 1891, belonging to the division Basidiomycota [15]. Furthermore, resting cells of the gram-positive bacterium *Nocardia asteroides* JCM3016 reduced benzoates to the corresponding alcohols [35]. Vanillic acid was reduced to vanillyl alcohol with another subspecies of *Nocardia*: *Nocardia iowensis* strain NRRL 5646 [42].

4 Aerobic carboxylate reductions by plants

Recently, lyophilized plant cell cultures were screened for their ability to reduce carboxylic acids and several plants, e.g. *Nicotiana tabacum* and *Helianthus annuus*, were able to reduce cinnamic, hexanoic, and octanoic acid to the corresponding aldehydes (in the case of three species) and alcohols in reduction yields from 2 to 80% w/w (Table 2, entry 1 and Table 5, entries: 21 and 29) [43].

5 Enzyme discovery and carboxylate reductions with purified and overexpressed enzymes

In contrast to the biotransformations using organisms, parts of organisms or cell extracts described in the previous sections, a range of aryl-aldehydes were prepared with a purified enzyme from *Neurospora crassa* – an aryl-aldehyde:NADP-oxidoreductase. This enzyme, isolated by Gross et al., was active for the reduction of cinnamic acid and its derivatives (Table 2). A first exploration of the reaction mechanism revealed that the enzyme was NADP(H) and ATP dependent and a positive influence of Mg²⁺ and dithiothreitol (DTT) on the enzyme's activity was found [44, 45]. These results were confirmed by the group of Rosazza and co-workers in 1997 with a homologous enzyme isolated from *N. iowensis* strain NRRL 5646,

Table 2. Cinnamic acid derivatives as substrates for carboxylate reducing activities

	R ¹	R ²	R ³	R ⁴	Biocatalyst	Refs.
1	H	H	H	H	<i>Corynespora cassiicola</i> IFO 7483 mycelium <i>Mucor</i> sp. JX23 growing cell culture/mycelium <i>Actinidia chinensis</i> ^{a), b), c)} , <i>Daucus carota</i> ^{a), c)} , <i>Helianthus annuus</i> ^{a), b), c)} , <i>Nicotiana tabacum</i> ^{a), b), c)} , <i>Polygonum persicaria</i> ^{a), b), c)} , <i>Rauwolfia manii</i> ^{a)} , <i>Solanum melanogeno</i> ^{b), c)} , and <i>Tagetes patula</i> ^{a), c)} Purified CAR from <i>Neurospora crassa</i> SY7A ^{d)} <i>Actinomyces</i> sp. ^{d)} <i>Pyrococcus furiosus</i> DSM 3638 growing cells Purified Mo-AOR from <i>Clostridium formicoaceticum</i> DSM 92 ^{e)} Partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^{e)} <i>Pyrococcus furiosus</i> DSM 3638 resting cells ^{h)}	[15] [37] [43] [44] [63] [71, 72] [60] [59] [74]
2	H	H	Cl	H	<i>Trametes versicolor</i> IFO 4941 mycelium	[15]
3	OMe	H	H	H	<i>Aspergillus flavus</i> DSM 1959 mycelium	[15]
4	H	OMe	OMe	H	<i>Trametes</i> sp. mycelium <i>Trametes hirsutus</i> IFO 4917 mycelium Purified CAR from <i>Neurospora crassa</i> SY7A ^{f)}	[16] [15] [44]
5	H	OCH ₂ O		H	Purified CAR from <i>Neurospora crassa</i> SY7A ^{f)}	[44]
6	H	OMe	H	OMe	<i>Aspergillus niger</i> ATCC 4192 mycelium	[15]
7	H	OMe	OMe	OMe	<i>Trametes</i> sp. mycelium Purified CAR from <i>Neurospora crassa</i> SY7A ^{f)}	[16] [44]
8	H	OMe	OH	H	<i>Trametes</i> sp. Mycelium <i>Mycobacterium phlei</i> DSM 43286 and <i>Psilocybe zapotekorum</i> DSM 1891 mycelium <i>Sporotrichum pulverulentum</i> ATCC 3262917 mycelium <i>Fomes fomentarius</i> mycelium Purified CAR from <i>Neurospora crassa</i> SY7A ^{f)} <i>Nocardia iowensis</i> sp. NRRL 5646 recombinant CAR heterologously expressed in <i>E. coli</i> and purified from it ^{f)} and resting <i>E. coli</i> cells overexpressing NiCAR	[15] [18] [17] [44] [47, 50]
9	H	OMe	OH	OMe	<i>Trametes</i> sp. mycelium	[16]
10	H	H	OMe	H	<i>Trametes</i> sp. mycelium <i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{f)}	[16] [46]
11	OH	H	H	H	<i>Corynespora cassiicola</i> CBS 12925 mycelium Purified CAR from <i>Neurospora crassa</i> SY7A ^{f)}	[15] [44]
12	H	OH	H	H	Purified CAR from <i>Neurospora crassa</i> SY7A ^{f)}	[44]
13	H	OH	OH	H	Purified CAR from <i>Neurospora crassa</i> SY7A ^{f)}	[44]
14	H	OH	OMe	H	Purified CAR from <i>Neurospora crassa</i> SY7A ^{f)}	[44]
15	H	H	OH	H	<i>Trametes</i> sp. mycelium <i>Fomes fomentarius</i> mycelium Purified CAR from <i>Neurospora crassa</i> SY7A ^{f)}	[16] [17] [44]

a) Resting cells from 7-day-old submerged cultures resuspended in buffer

b) Resting cells from 7-day-old submerged cultures, lyophilized and resuspended in buffer

c) Resting cells from 7-day-old submerged cultures, lyophilized and resuspended in biphasic system

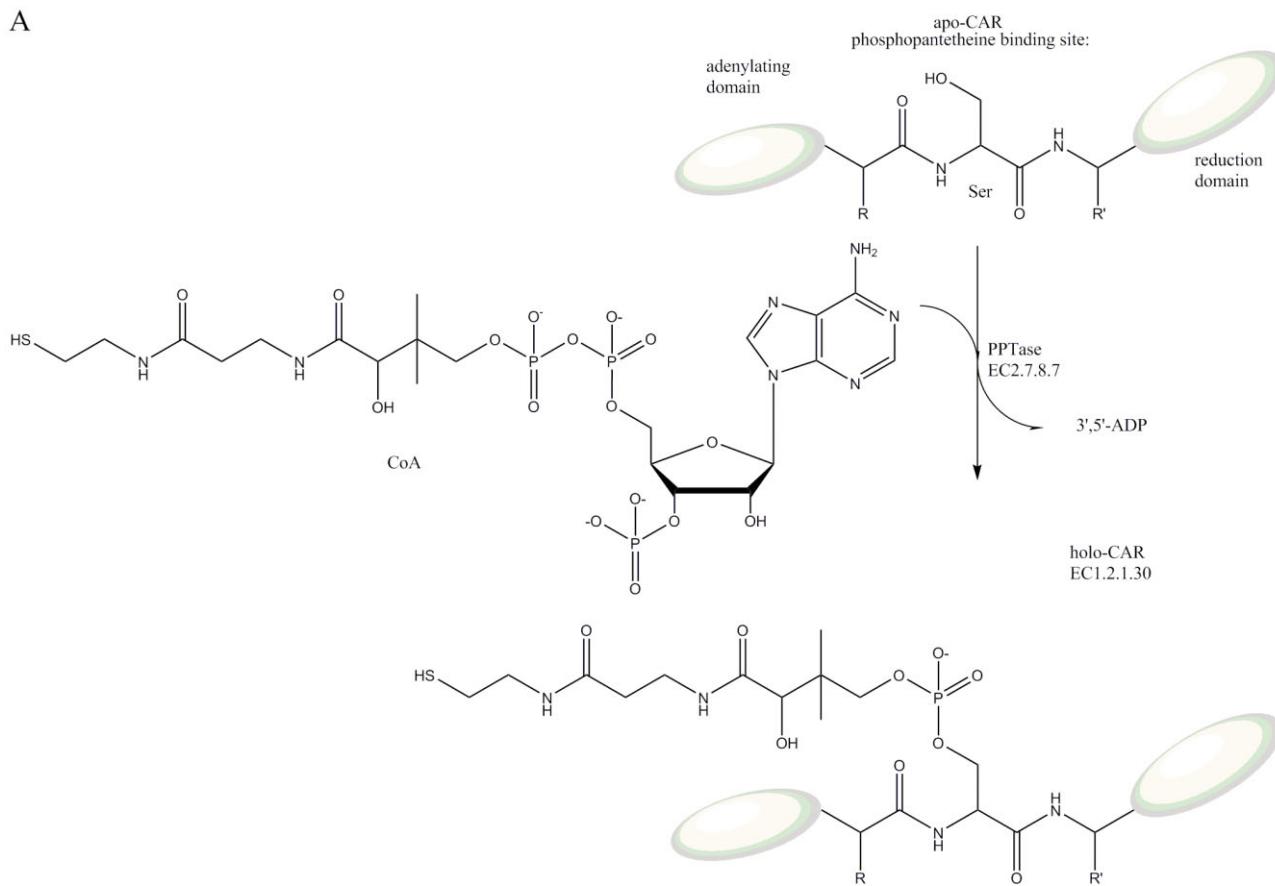
d) Lyophilized and permeabilized whole cells

e) In the presence of CAV⁺⁺

f) In the presence of ATP and NADPH

g) In the presence of TMV⁺⁺h) In the presence of H₂ or CO

A



B

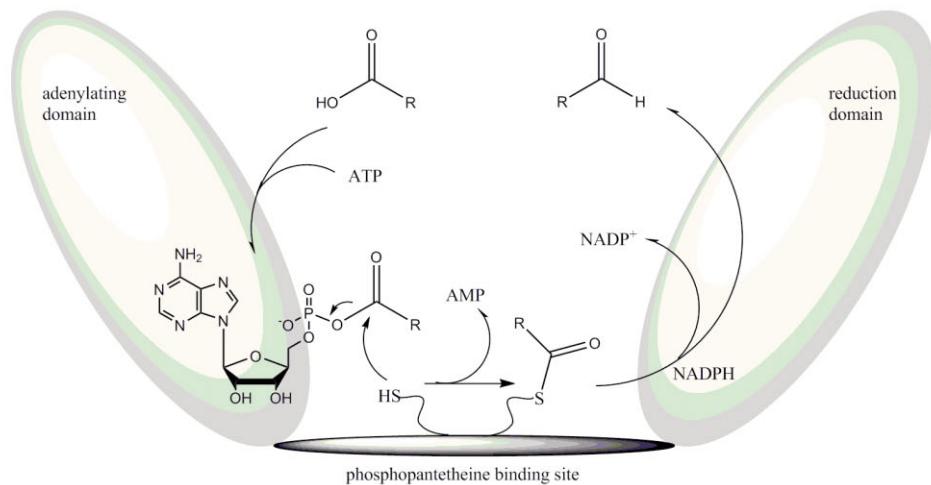


Figure 2. (A) Post-translational modification of carboxylate reductases. Transfer of a phosphopantetheine moiety from the donor (CoA) to the conserved serine residue of apo-CAR catalyzed by PPTase. PPT-binding site motif: LGG-x-S-xx-A. (B) Proposed catalytic cycle of holo-CAR reduction of benzoic acid to benzaldehyde. Deprotonated carboxylic acid is activated as adenosyl phosphate at the adenylating domain at the expense of ATP. This, in turn, is nucleophilically attacked by the phosphopantetheine thiol and forms a covalently bound thioester with release of AMP. The thioester is finally reduced at the reduction domain to aldehyde at the expense of NADPH; (adapted from [48]).

Table 3. Phenylacetic acid derivatives as substrates for carboxylate reducing activities

	R ¹	R ²	R ³	R ⁴	Biocatalyst	Refs.
1	H	H	H	H	<i>Nocardia asteroides</i> JCM 3016 resting cells <i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)} <i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{b)} <i>Clostridium formicoaceticum</i> DSM 92 crude extract ^{c)}	[35, 36] [46] [59] [56]
2	OMe	H	H	H	<i>Trametes hirsutus</i> IFO 4917 mycelium	[15]
3	H	H	H	OMe	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{b)}	[59]
4	H	OH	OH	H	<i>E. coli</i> resting cells heterologously expressing recombinant NiCAR from <i>Nocardia iowensis</i> sp. NRRL 5646	[52]
5	H	H	H	OH	<i>Nocardia asteroides</i> JCM 3016 resting cells	[35, 36]
6	NO ₂	H	H	H	<i>Trametes versicolor</i> IFO 4941 mycelium	[15]
7	H	NO ₂	H	H	<i>Trametes versicolor</i> IFO 4941 mycelium	[15]
8	H	H	H	Me	<i>Glomerella cingulata</i> (<i>Prunus</i> and <i>Malus</i> strains), <i>Gloeosporium olivarum</i> and <i>Gloeosporium laeticolor</i> growing cell culture <i>Nocardia asteroides</i> JCM 3016 resting cells <i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)} <i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{b)} <i>Clostridium formicoaceticum</i> DSM 92 crude extract ^{c)} <i>Clostridium thermoaceticum</i> resting cells ^{b)}	[39, 40] [35, 36] [46] [59] [56] [55]
9	H	H	H	COOH	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[46]
10	H	H	H	CH ₂ COOH	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[46]
11	H	H	iBu	Me (R)	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[46]

a) In the presence of ATP and NADPH

b) In the presence of CO and MV

c) In the presence of CO

a gram-positive bacterium. They purified and characterized a carboxylate reductase from *Nocardia* sp. NRRL 5646 (NiCAR) and investigated 58 substrates, mostly benzoic acid and its halogen, methoxy, acetyl, nitrile, phenyl, phenoxy, and benzoyloxy derivatives as well as toluic, anisic (Table 1), naphthoic, furoic, nicotinic (Table 4), phenylmalonic, -succinic, -acetic (Table 5) and 2-phenylpropionic acid (Table 3). From all substrates, 3-chlorobenzoic acid was superior to 3-bromobenzoic, 4-chlorobenzoic, and benzoic acid. In general, 3-substituted benzoic acids were converted best, whereas substitutions in 2-position resulted in poor conversions. For all variants of nitrobenzoic acids, NiCAR did not show any activity. Due to this substrate scope, the enzyme was classified as aryl-aldehyde dehydrogenase (NADP⁺) with the E.C. number 1.2.1.30. An investigation with ibuprofen as a substrate showed that the enzyme was (R)-selective [46]. In 2000, Rosazza and co-workers [42] found that vanillic acid was reduced to vanillyl alcohol with *Nocardia* resting cells, whereas with the purified enzyme the reduction evidently stopped at the stage of the aldehyde vanillin: an indication that *Nocardia* cells have to have

one or more dehydrogenases responsible for further reduction of vanillin to vanillyl alcohol. In 2004, the gene coding for the NiCAR protein was discovered and cloned for heterologous expression in *E. coli* [47], however, a functional protein was only obtained after a post-translational phosphopantetheinylation. Specifically, the 4-phosphopantetheine moiety of CoA is covalently attached to a conserved serine residue of the CAR apoprotein and thereby it is converted into the active holoprotein (Fig. 2A) [48]. The reaction is catalyzed by a phosphopantetheinetransferase (PPTase), the role of which was previously documented in non-ribosomal peptide and fatty acid synthesis [49].

Venkitasubramanian et al. elucidated the catalytic function of the carboxylate reductase enzyme, discovered that 3 domains are necessary for catalysis and suggested a reaction mechanism (Fig. 2B) [48]:

- Adenylylating domain to activate the free, deprotonated carboxylic acid as an adenosyl phosphate at the expense of ATP;
- Phosphopantethein binding site (which needs the additional PPTase to attach phosphopantethein at the

Table 4. Poly- and heterocyclic carboxylic acids as substrates for carboxylic acid reducing activities

Compound structure	Biocatalyst	Refs.
1	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[46]
2	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)} <i>Trametes versicolor</i> mycelium	[46] [13]
3	<i>Hebeloma sacchariolens</i> fruit bodies and homogenate	[32]
4	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[46]
5	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[46]
6	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[46]
7	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[46]
8	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)}	[46]
9	Partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^{a)}	[59]
10	<i>Hebeloma sacchariolens</i> fruit bodies and homogenate	[32]
11	<i>Hebeloma sacchariolens</i> fruit bodies and homogenate	[32]
12	<i>Nocardia iowensis</i> sp. NRRL 5646 crude extract ^{a)} Partially purified W-AOR from <i>Clostridium thermoaceticum</i> ^{b)}	[46] [59]
13	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{c)} and partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 521 ^{b)}	[59]

Table 4. Poly- and heterocyclic carboxylic acids as substrates for carboxylic acid reducing activities (continued)

Compound structure	Biocatalyst	Refs.
14 	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{c)}	[59]
15 	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{c)} Purified Mo-AOR from <i>Clostridium formicoaceticum</i> DSM 92 in the presence of TMV ⁺	[59] [60]

a) In the presence of ATP and NADPH
b) In the presence of CAV⁺⁺
c) In the presence of CO and MV

expense of CoA to the enzyme, Fig. 2A) that attacks the carbonyl of the activated ester to form a thioester;

- Reduction domain that reduces the thioester and releases an aldehyde.

Combining mechanistic and sequence information of the NiCAR enzyme with previous results of vanillic acid reduction, NiCAR was expressed in *E. coli*. In addition to the NiCAR sequence, the plasmid carried also the *Bacillus subtilis* PPTase and glucose dehydrogenase for efficient cofactor recycling [50] to improve vanillic acid reduction further [42]. Alternatively, Hansen et al. used *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* to generate microbial strains for the production of vanillin. The required phosphopantetheinylation was achieved by coexpression of PPTase from *Corynebacterium glutamicum*. The overreduction to vanillyl alcohol by host enzymes was overcome by the knockout of the host alcohol dehydrogenase *ADH6* [51]. In this study, an obstacle, the over-reduction was an unexpected lucky chance in another application of the NiCAR enzyme: 3,4-dihydroxyphenylacetic acid was reduced to the antioxidant 3-hydroxytyrosol by resting *E. coli* cells co-expressing NiCAR and the PPTase from *E. coli* (Table 3, entry 4) [52].

The substrate scope of the NiCAR enzyme was further enlightened with a purified enzyme preparation. Several non-aromatic carboxylic mono- and diacids were tested as substrates making use of the photometric detection of NADPH consumption. Although 2-ketoglutaric acid appeared to be the best substrate [50] it needs to be stressed that a comparison of mono- and diacids in a cofactor based activity assay is difficult, because no information may be gained whether only one or both acid functionalities are being reduced. With respect to aliphatic compounds, NiCAR was also considered as a catalyst for fatty acid reduction [53], however, a homologous protein from *Mycobacterium marinum* (*MmCAR*), sharing 62% identity with NiCAR proved capable of reducing C₆-C₁₈ fatty acids to the respective aldehydes. With this carboxylate reduction as the key step, cell factories expressing a pathway of four

exogenous enzymes (e.g. the aldehyde reductase from *Synechocystis* sp. PCC 6803) were developed for the preparation of fatty alcohols [54].

6 Anaerobic carboxylate reductions by bacteria

In addition to carboxylate reductase activities described in the previous chapters, microbial reductions of carboxylic acids under anaerobic conditions have been reported. To our knowledge, the first publication concerning this type of reaction appeared in 1987. In the presence of CO, H₂ or formate, methyl viologen (MV) and whole cells of *Clostridium thermoaceticum*, a range of aliphatic and aromatic carboxylic acids were reduced to the respective alcohols [3], among which hexanedioic acid appeared to be the best substrate. It was reasoned that if the redox potential of carbon monoxide dehydrogenase from *C. thermoaceticum* had been quoted for -560 mV, whereas the redox potential of carboxylate/aldehyde had been estimated for -550 mV, this microbe should be able to reduce carboxylic acids. However, the addition of methyl viologen as a redox mediator proved to be essential to promote this reaction [55]. Crude extract or supernatant of broken cells of *Clostridium formicoaceticum* DSM 92 was reported to reduce branched and unbranched, saturated and unsaturated carboxylates at the expenses of CO to the corresponding alcohols. Interestingly, in contrast to *C. thermoaceticum* DSM 521, it was found that the reduction of carboxylates with *C. formicoaceticum* DSM 92 proceeded faster in the absence of viologens [56]. The enzymes responsible for carboxylic acid reduction in these two organisms were isolated and characterized to be highly oxygen sensitive tungsten enzymes (W-AOR, carboxylate reductases, E.C.1.2.99.6) [57–59], however, also a less oxygen sensitive molybdenum containing aldehyde oxidoreductase (Mo-AOR) was identified [60]. Similar to the NiCAR enzyme, both purified CfAORs reduced carboxylic acids selectively to aldehydes, despite the apparent differences of their catalytic mechanism, where-

Table 5. Aliphatic carboxylic acids and derivatives thereof as carboxylate reductase substrates

Compound structure	Biocatalyst	Refs.
1 	<i>Clostridium thermoaceticum</i> resting cells ^{a)} <i>Clostridium formicoaceticum</i> DSM 92 crude extract ^{b)} Purified W-AOR and Mo-AOR from <i>Clostridium formicoaceticum</i> DSM 92 ^{c)} <i>Clostridium ljungdahlii</i> ERI-2 (ATTC 55380) and <i>Clostridium ragsdalei</i> P11 (ATTC BAA-622) ^{d)} Mixed culture from anaerobic granular sludge Mixed culture of anaerobic sulfate reducing bacteria <i>Thermococcus</i> sp.1 ES-1 in the presence of MV ⁺	[55] [56] [57,60] [61] [68,69] [70] [78]
2 	<i>Clostridium thermoaceticum</i> DSM 92 resting cells ^{a)} Partially purified W-AOR from <i>Clostridium thermoaceticum</i> DSM 92 ^{e)} or in the presence MV ⁺ <i>Clostridium formicoaceticum</i> crude extract ^{b)} Purified W-AOR and Mo-AOR from <i>Clostridium formicoaceticum</i> ^{c)} <i>Clostridium ljungdahlii</i> ERI-2 (ATTC 55380) and <i>Clostridium ragsdalei</i> P11 (ATTC BAA-622) ^{d)} Mixed culture from anaerobic granular sludge <i>Pyrococcus furiosus</i> DSM 3638 growing cells	[55] [34,61] [56] [57,60] [61] [68] [72]
3 	<i>Clostridium ljungdahlii</i> ERI-2 (ATTC 55380) and <i>Clostridium ragsdalei</i> P11 (ATTC BAA-622) ^{d)}	[61]
4 	<i>Nocardia asteroides</i> JCM 3016 resting cells <i>Pyrococcus furiosus</i> DSM 3638 growing and resting cells ^{e)}	[35,36] [71–73]
5 	<i>Clostridium thermoaceticum</i> resting cells ^{a)}	[55]
6 	<i>Clostridium thermoaceticum</i> resting cells ^{a)} <i>Clostridium formicoaceticum</i> crude extract ^{b)} Purified Mo-AOR from <i>Clostridium formicoaceticum</i> DSM92 ^{c)} <i>Clostridium ljungdahlii</i> ERI-2 (ATTC 55380) and <i>Clostridium ragsdalei</i> P11 (ATTC BAA-622) ^{d)} <i>Clostridium saccharoperbutylacetonicum</i> strain N1-4 (ATCC 27021) slowly growing cells Mixed culture from anaerobic granular sludge Mixed culture of anaerobic sulfate reducing bacteria <i>Pyrococcus furiosus</i> DSM 3638 growing and resting cells ^{e)}	[55,59] [56] [60] [61] [62] [68] [70] [71–73]
7 	<i>Pyrococcus furiosus</i> DSM 3638 growing cells	[72]
8 	<i>Clostridium thermoaceticum</i> resting cells ^{a)}	[55]
9 	<i>Pyrococcus furiosus</i> DSM 3638 resting cells ^{e)}	[73]
10 	<i>Clostridium thermoaceticum</i> DSM 521resting cells ^{a)}	[59]
11 	<i>Clostridium thermoaceticum</i> DSM 521resting cells ^{a)}	[55, 59]

Table 5. Aliphatic carboxylic acids and derivatives thereof as carboxylate reductase substrates (continued)

Compound structure	Biocatalyst	Refs.
12	<i>Actinomyces</i> sp. ^{f)} Purified Mo-AOR from <i>Clostridium formicoaceticum</i> DSM 92 ^{c)} <i>Pyrococcus furiosus</i> DSM 3638 growing and resting cells ^{b)}	[63] [60] [72, 74]
13	<i>Clostridium thermoaceticum</i> resting ^{a)}	[55]
14	<i>Clostridium thermoaceticum</i> resting cells ^{a)} <i>Clostridium formicoaceticum</i> DSM 92 crude extract ^{b)}	[55] [56]
15	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{a)} <i>Clostridium formicoaceticum</i> DSM 92 crude extract ^{b)} <i>Clostridium ljungdahlii</i> ERI-2 (ATTC 55380) and <i>Clostridium ragsdalei</i> P11 (ATTC BAA-622) ^{d)} <i>Pyrococcus furiosus</i> DSM 3638 resting cells ^{e)}	[59] [56] [61] [73]
16	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{a)}	[59]
17	<i>Pyrococcus furiosus</i> DSM 3638 growing cells	[72]
18	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{a)}	[59]
19	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{a)}	[59]
20	<i>Pyrococcus furiosus</i> DSM 3638 resting cells ^{e)}	[73]
21	<i>Clostridium formicoaceticum</i> resting cells ^{b)} <i>Actinidia chinensis</i> ^{h)} , <i>Daucus carota</i> ^{h),i)} , <i>Helianthus annuus</i> ^{h),i),j)} , <i>Nicotiana tabacum</i> ^{h),i),j)} , <i>Phytolacca decandra</i> ^{g)} , <i>Polygonum persicaria</i> ^{h)} , <i>Rauvolfia manii</i> ^{h)} , <i>Solanum melanogenae</i> ^{h),i),j)} , and <i>Tagetes patula</i> ⁱ⁾ <i>Clostridium ljungdahlii</i> ERI-2 (ATTC 55380) and <i>Clostridium ragsdalei</i> P11 (ATTC BAA-622) ^{d)} <i>Pyrococcus furiosus</i> DSM 3638 growing and resting cells ^{e)}	[56] [43] [61] [71–73]
22	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{a)}	[59]
23	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{a)}	[59]
24	<i>Pyrococcus furiosus</i> DSM 3638 growing cells ^{e)}	[72]
25	<i>Pyrococcus furiosus</i> DSM 3638 resting cells ^{b)}	[74]
26	<i>Botrytis cinerea</i> 5889/4, 5901/2, 5882/1, and 5909/1 mycelium	[41]
27	<i>Pyrococcus furiosus</i> DSM 3638 resting cells ^{e)}	[73]
28	<i>Pyrococcus furiosus</i> DSM 3638 resting cells ^{e)}	[73]

Table 5. Aliphatic carboxylic acids and derivatives thereof as carboxylate reductase substrates (continued)

Compound structure	Biocatalyst	Refs.
29	<i>Clostridium thermoaceticum</i> resting cells ^{a)}	[55]
30	<i>Clostridium formicoaceticum</i> DSM 92 crude extract ^{b)} Purified Mo-AOR from <i>Clostridium formicoaceticum</i> DSM 92 ^{c)} <i>Actinidia chinensis</i> , <i>Daucus carota</i> , <i>Helianthus annuus</i> , <i>Phytolacca decandra</i> , <i>Polygonum persicaria</i> , <i>Rauwolfia manii</i> , <i>Solanum melanogena</i> , and <i>Tagetes patula</i> ^{h)}	[56] [60] [43]
31	<i>Botrytis cinerea</i> 5889/4, 5901/2, 5882/1, and 5909/1 mycelium	[41]
32	<i>Clostridium formicoaceticum</i> DSM 92 crude extract ^{b)} <i>Pyrococcus furiosus</i> DSM 3638 growing and resting cells ^{e)}	[56] [72, 73]
33 Fatty acids C ₆ -C ₁₈	CAR from <i>Mycobacterium marinum</i> overexpressed in <i>E. coli</i>	[54]
34 Fatty acids C ₁₂ -C ₁₈ (saturated and unsaturated)	Growing cells of <i>E. coli</i> expressing fatty acyl-ACP reductase from <i>Synechococcus elongatus</i> Growing cells of <i>E. coli</i> expressing fatty acyl-CoA reductase from <i>Arabidopsis thaliana</i>	[80] [85]
35 Fatty acids C ₁₄ -C ₂₂ (saturated) and Fatty acids C ₁₆ -C ₂₀ (unsaturated)	Growing cells of <i>S. cerevisiae</i> expressing fatty acyl-CoA reductase from <i>Apis mellifera</i>	[86]
36 Myristic acid (C ₁₄)	<i>Photobacterium phosphoreum</i> transferase, synthetase and reductase <i>Pyrococcus furiosus</i> DSM 3638 growing cells Growing cells of <i>S. cerevisiae</i> expressing fatty acyl-CoA reductase from <i>Euglena gracilis</i>	[79] [72] [87]
37 Palmitic acid (C ₁₆)	<i>Pyrococcus furiosus</i> DSM 3638 growing cells Growing cells of <i>S. cerevisiae</i> expressing fatty acyl-CoA reductase from <i>Euglena gracilis</i>	[72] [87]
38	<i>Nocardia asteroides</i> JCM 3016 resting cells	[35, 36]
39	<i>Nocardia iowensis</i> sp. NRRL 5646 ⁱ⁾ <i>Clostridium thermoaceticum</i> resting cells ^{a)}	[50] [55]
40	<i>Glomerella cingulata</i> (<i>Prunus</i> and <i>Malus</i> strain), <i>Gloeosporium olivarum</i> and <i>Gloeosporium laeticolor</i> growing cell culture	[38–40]
41	<i>Streptomyces lavendulae</i> SfmC, purified and treated with PPTase ^{k)}	[84]
42	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{a)}	[59]
43	<i>Gloeosporium olivarum</i> growing cell culture <i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{a)}	[39] [59]

Table 5. Aliphatic carboxylic acids and derivatives thereof as carboxylate reductase substrates (continued)

Compound structure	Biocatalyst	Refs.
44	<i>Gloeosporium olivarum</i> growing cell culture <i>Clostridium thermoaceticum</i> resting cells ^{a)}	[39] [59]
45	<i>Gloeosporium olivarum</i> growing cell culture	[39]
46	<i>Gloeosporium olivarum</i> growing cell culture <i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{a)}	[39] [59]
47	<i>Gloeosporium olivarum</i> growing cell culture	[39]
48	<i>Gloeosporium olivarum</i> growing cell culture	[39]
49	<i>Glomerella cingulata</i> (<i>Prunus</i> and <i>Malus</i> strain), <i>Gloeosporium olivarum</i> and <i>Gloeosporium laeticolor</i> growing cell culture	[40]
50	<i>Glomerella cingulata</i> (<i>Prunus</i> and <i>Malus</i> strain), <i>Gloeosporium olivarum</i> and <i>Gloeosporium laeticolor</i> growing cell culture	[40]
51	<i>Gloeosporium olivarum</i> growing cell culture	[39]
52	<i>Pyrococcus furiosus</i> DSM 3638 resting cells ^{e)}	[73]
53	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{a)}	[55, 59]
54	<i>Nocardia iowensis</i> sp. NRRL 5646 ⁱ⁾	[50]
55	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{a)}	[59]
56	<i>Nocardia iowensis</i> sp. NRRL 5646 ^{k,l)}	[50]
57	<i>Nocardia iowensis</i> sp. NRRL 5646 ^{k,l)}	[50]

Table 5. Aliphatic carboxylic acids and derivatives thereof as carboxylate reductase substrates (continued)

Compound structure	Biocatalyst	Refs.
58	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{a)}	[55, 59]
59	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{a)}	[59]
60	<i>Nocardia iowensis</i> sp. NRRL 5646 ^{k),l)}	[50]
61	<i>Nocardia iowensis</i> sp. NRRL 5646 ^{k),l)}	[50]
62	<i>Nocardia iowensis</i> sp. NRRL 5646 ^{k),l)}	[50]
63	<i>Nocardia iowensis</i> sp. NRRL 5646 ^{k),l)}	[50]
64	<i>Nocardia iowensis</i> sp. NRRL 5646 ^{k),l)}	[50]
65	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{a)}	[55, 59]
66	<i>Clostridium thermoaceticum</i> DSM 521 resting cells ^{a)}	[59]
67	<i>Schizosaccharomyces pombe</i> wt 972, cell free extract, <i>Schizosaccharomyces pombe</i> Lys1p expressed in <i>E. coli</i> and <i>Candida albicans</i> Lys2p expressed in <i>E. coli</i>	[81, 82]

a) In the presence of CO and MV

b) In the presence of CO

c) In the presence of TMV⁺

d) Growing cells with syngas

e) In the presence of H₂

f) In the presence of C₆V⁺

g) Lyophilized and permeabilized whole cells

h) Resting cells from 7-day-old submerged cultures resuspended in buffer

i) Resting cells from 7-day-old submerged cultures, lyophilized and resuspended in buffer

j) Resting cells from 7-day-old submerged cultures, lyophilized and resuspended in biphasic system

k) In the presence of ATP and NADPH

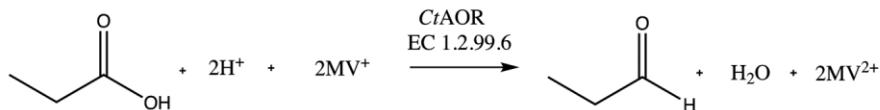
l) Recombinant CAR heterogeneously expressed in *E. coli* and purified from it

as whole cell bioconversions catalyzed by *Clostridia* typically yield alcohols [55].

Perez et al. [61] reported the use of syngas in combination with whole cells of *Clostridium ljungdahlii* and *Clostridium ragsdalei* for reducing additional aliphatic carboxylic acids such as acetic, propionic, *n*-butyric, *n*-valeric, and *n*-caproic acid to the corresponding alcohols (Table 5, entries: 1, 2, 6, 15, and 21). Syngas is a synthetic

mixture of CO, CO₂, and H₂, generated during thermal pyrolysis of lignocelluloses. Hence, the idea was to apply carboxydrophic bacteria that consume easily available syngas and produce valuable biofuels such as *n*-butanol [61]. Another environmental-friendly route to *n*-butanol was in the spotlight of Richter et al. They reduced *n*-butyrate from lignocellulose to *n*-butanol with the solventogenic *Clostridium saccharoperbutylacetonicum* strain

A



B

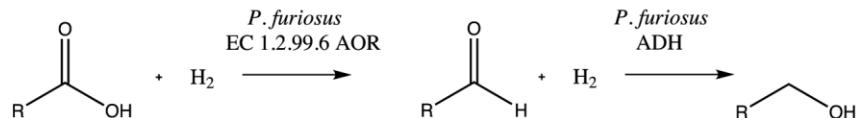


Figure 3. (A) Carboxylic acid reduction catalyzed by tungsten enzyme E.C.1.2.99.6. Non-activated acid is reduced by reduced viologens to aldehydes (adapted from [58]). (B) *Pyrococcus furiosus* catalyzed hydrogenation of carboxylic acids. Hydrogen is oxidized by an internal oxidase, whereas carboxylic acid is reduced by an oxygen sensitive tungsten pterin enzyme. Aldehyde product is further reduced to the respective alcohol by endogenous reductase activity (adapted from [73]).

N1-4 (ATCC 27021). Glucose (60 g/L) served as a carbon source for cell growth and as a source for ATP and reducing equivalents [62].

Aromatic carboxylic acids were reported as substrates of another anaerobic bacteria species: *Actinomyces* sp. The reaction gave the corresponding aldehydes in yields from 5 to 67%. Since the aldehyde products were detected via their 2,4-dinitrophenylhydrazones, further reduction to alcohol may not be excluded. The *Actinomyces* sp. cells were lyophilized and additionally permeabilized with toluene in order to allow substrate access. Derivatives of acrylic acid such as cinnamic and crotonic acid were converted sluggishly in comparison to the best substrates vanillic acid and 3,4,5-trimethoxybenzoic acid [63].

The Desulfovibrionales species also exhibits carboxylic acid reducing activity under anaerobic conditions. A culture of *Desulfomicrobium escambiense* reduced 3-chloro and 3-bromobenzoate to the corresponding benzyl alcohols in the presence of pyruvate [64]. Interestingly, these compounds were not converted by *Desulfovibrio vulgaris* PY1, which reduced nine other halogenated benzoic acids under the same conditions. However, when MV, FAD, FMN, or ferredoxin as electron carrier and pyruvate or H₂ as electron donor were supplied to cell free extracts of this organism, 3-chlorobenzoate could be reduced as well. Also reduction of the sulfate content of the medium allowed this conversion [65].

Mixed anaerobic cultures enjoy increasing interest, because of their potential use for the conversion of waste materials to bioproducts, especially within the so-called “carboxylate platform.” This term refers to carboxylates generated with undefined mixed cultures from organic feedstocks derived from industrial and agricultural wastes. During primary fermentation, acetate, propionate, lactate and n-butyrate are generated and subsequently proceeded (*inter alia* reduced to the alcohols) during secondary fermentation [66]. Technical and economic analysis of such processes as well as the aforementioned syngas fermentation showed that both methods are worth

to be commercialized [67]. Such mixed cultures were, e.g. used for the production of ethanol, propanol, and n-butanol from acetate, propionate, and n-butyrate, respectively. Specifically, granular sludge from up-flow anaerobic sludge blanket reactors was used for this purpose. All three carboxylic acids, as well as the hydrogen needed as an electron donor, were derived from fermentative waste biomass acidification and the respective alcohols represented about 50% of the obtained products, whereas the by-products were dominated by methane (30%) [68]. Alternatively, hydrogen was replaced with an electrode as electron donor and MV⁺ was used to accelerate acetate reduction [69]. In a similar approach, Sharma et al. recently used a mixed culture of sulfate-reducing bacteria for bio-electrocatalyzed reduction of acetic and butyric acid via direct electron transfer. The products of this reduction were mainly ethanol and butanol, although also caproate and acetone were detected [70].

7 Anaerobic carboxylate reductions by Archaea

Also hyperthermophilic archaea such as *Pyrococcus furiosus* DSM 3638 exhibit reductive activities towards carboxylic acid substrates [71]. It was shown that *P. furiosus* batch cultures at anaerobic conditions and 90°C reduced trans-cinnamic acid and 3-phenylpropionic acid to the corresponding alcohols in 67 and 69% conversion, respectively. Among aliphatic acids, the best accepted substrate was hexanoic acid with a reduction yield of 38% to hexanol [72]. In another study, Ni et al. also used *P. furiosus* DSM 3638 as the hydrogenating biocatalyst, which can combine H₂ oxidation (catalyzed by a hydrogenase) with carboxylic acid reduction to aldehydes (catalyzed by an aldehyde ferredoxin reductase – AOR) and further to alcohols (mediated by endogenous ADH) (Fig. 3B). Eleven carboxylic acids were reduced to alcohols in up to 99% conversion. Medium-chain aliphatic acids were reduced

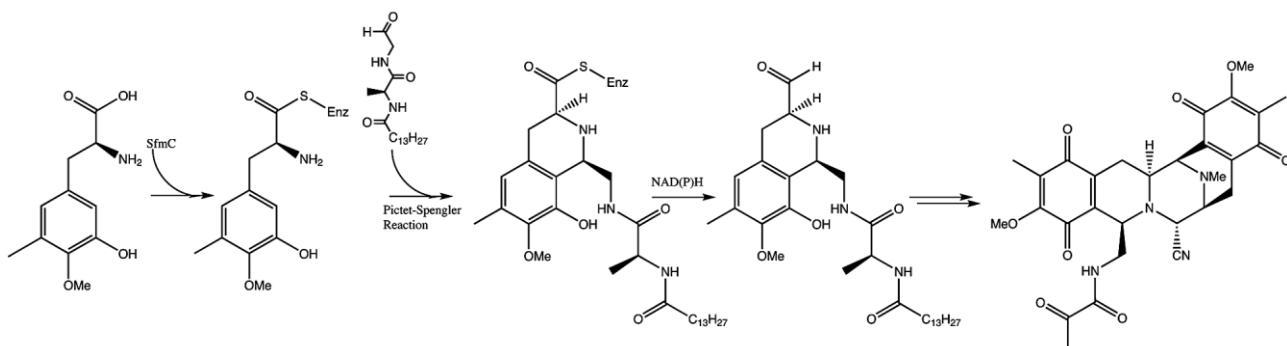


Figure 4. Carboxylate reduction in Saframycin biosynthesis. In the proposed reaction pathway, SfmC binds the tyrosin derivative as thioester. Then, a Pictet-Spengler reaction between the amine group and an aldehyde affords the isoquinoline intermediate. Subsequently, the thioester is reduced to an aldehyde intermediate, which is converted in several additional steps to Saframycin (adapted from [84]).

within 24 h whereas the reaction rate for longer chain aliphatic acids was slower. Hydrogen (H_2) that is oxidized by a hydrogenase enzyme in this organism, serves as reducing equivalent and the carboxylate reduction itself is catalyzed by an oxygen sensitive tungsten pterin enzyme [73]. Further experiments from the same research group showed that also CO can be used as a much cheaper reductant than H_2 [74] as it was also shown by Perez et al. [61] for *Clostridia* strains. Product distribution of cinnamic acid reduction was almost equal, regardless of the reductant, but in case of α,β -unsaturated carboxylic acids both the reduction of carbonyl and C=C groups was observed, resulting in target product yields <40% [74]. These undesired side reactions were not observed, when solely H_2 was used [73]. A particular challenge in the reduction of carboxylic acids and their derivatives is the selective termination of the reduction process at the stage of the aldehyde. In *P. furiosus* catalyzed bioreductions, simple medium engineering led to the success: specifically, the aqueous reaction medium was replaced by hexadecane. This substitution allowed for aldehyde accumulation, although the overall activity decreased [73]. The enzyme catalyzing the above mentioned carboxylic acid reduction was isolated already two decades before by Mukund and Adams [75] and was initially described as red-colored tungsten-containing protein (RTP). However, further investigation showed that RTP is in fact an inac-

tive form of an aldehyde ferredoxin reductase (AOR), which, in contrast to RTP, contains a catalytically essential W-SH group [76]. The other four members of AOR family enzymes do not reduce carboxylic acids [77]. Another tungsten-containing AOR, also from a hyperthermophilic archaeon – *Thermococcus ES-1* – showed high homology to *P. furiosus* AOR and *C. formicoaceticum* AOR and the enzyme itself was able to reduce acetate at 85 °C with methyl viologen (MV^+) as the electron donor, although the observed activity was much lower than the reverse aldehyde oxidation [78].

8 Other examples of carboxylate-reducing enzymatic activities

Biocatalytic reduction of carboxylic acids is also well known from bacterial bioluminescence, where the long chain aldehydes that are required for the luminescent reaction are generated by fatty acid reduction ([79], and references therein) and fatty acid biosynthesis ([80], and references therein). Also in one of the biosynthetic pathways of lysine in fungi, the substrate α -amino adipic acid is reduced to α -amino adipate- δ -semialdehyde by α -amino adipate reductase (E.C.1.2.1.31) [81, 82]. This enzyme class appears to be highly substrate specific, as no other substrate was reported to date.

Table 6. Enzyme classes catalyzing carboxylic acid reduction

Enzyme class	Names	Common abbreviation
E.C. 1.2.1.30	aryl-aldehyde dehydrogenase ($NADP^+$), aromatic acid reductase	CAR (carboxylic acid reductase)
E.C. 1.2.1.31	L-amino adipate-semialdehyde dehydrogenase	AAR (α -amino adipate reductase)
E.C. 1.2.1.42	Hexadecanal dehydrogenase (acylating)	FAR (fatty acyl-CoA reductase)
E.C. 1.2.1.50	Long-chain-fatty-acyl-CoA reductase	FAR (fatty acyl-CoA reductase)
E.C. 1.2.1.80	Long-chain acyl-[acyl-carrier-protein] reductase	AAR (fatty acyl-ACP reductase)
E.C. 1.2.1.84	Alcohol-forming fatty acyl-CoA reductase	FAR (fatty acyl-CoA reductase)
E.C. 1.2.99.6	Carboxylate reductase, aldehyde oxidoreductase	AOR (aldehyde ferredoxin oxidoreductase)

An enzyme possessing carboxylic acid reduction ability has been found in the course of antibiotic biosynthesis in *Streptomyces griseus*. The protein products of *griC* and *griD* catalyze the ATP and NADPH dependent reduction of 3-amino-4-hydroxybenzoic acid to its aldehyde which serves as the precursor of grixazone A (one of two ingredients of a yellow pigment produced by *S. griseus*) [83]. In a similar report, the non-ribosomal polypeptide synthetase SfmC from *Streptomyces lavendulae* catalyzed the reduction of the carboxylate from a tyrosine derivative (Table 5, entry 40), to its aldehyde as the key step to the antitumor antibiotic Saframycin A (Fig. 4) [84].

Summarizing, three enzyme classes catalyze the reduction of free carboxylic acids to aldehydes. E.C. 1.2.99.6 consists of oxygen sensitive metal dependent proteins that were not extensively studied or used for biocatalysis so far. E.C. 1.2.1.30 and 31 are proteins, consisting of NADP⁺ dependent aryl-aldehyde dehydrogenase and α -aminoacidate reductase. In addition, non-ribosomal peptide synthetases may catalyze carboxylate reductions. From fatty acid biosynthesis and bioluminescence, fatty acyl-ACP reductases and fatty acyl-CoA reductases are known, which formally also reduce acids, however, bound to high molecular weight residues (Table 6). Therefore, these latter enzymes have not been reviewed in detail here.

9 Conclusions

Compared to the vast majority of chemical routes towards the reduction of carboxylic acids, carboxylate reductase enzymes allow for catalytic processes with perfect chemoselectivity: functional groups other than carboxylic acids remain unaffected. Most importantly, the aldehyde product is not reduced by these enzymes further, as proven in a number of biochemical studies with purified enzymes. In potential applications with, e.g. whole cell systems, the challenge remains to trap the valuable, typically rather reactive aldehyde products from further reactions. Although many potential sources for carboxylate reductases were described, only a handful of gene and/or protein sequences are currently known. The lion's share of these enzymes remains to be explored and exploited for biotechnology. From the industrial viewpoint, the selectivity of carboxylate reducing enzymes is a highly interesting feature, however, the technology development is still in its infancy. Although, e.g. the production of *n*-butanol and other biofuels looks promising for commercialization, improvement is still necessary to compete in the bulk-sector. It may be more likely that CAR enzymes will be implemented to produce food/feed ingredients or contribute in reaction cascades towards pharma products in the near future.



Kamila Napora-Wijata recently received her PhD in Biotechnology from Graz University of Technology (Austria). She studied Biotechnology at Lodz University of Technology (Poland). In 2010, she started her PhD at the Austrian Centre of Industrial Biotechnology (ACIB GmbH) under supervision of Prof. Peter Macheroux. Her project was focused on the identification and expression of new microbial oxidoreductases. Her research interests are biocatalysis and protein expression optimization.



Gernot A. Strohmeier studied chemistry and business administration and obtained his PhD in chemistry from the University of Graz in 2003 for research on combinatorial chemistry. In 2005 he joined the Applied Biocatalysis Research Centre in Graz and started with research on biocatalytic applications for the synthesis of chiral compounds and pharmaceuticals. He is currently a researcher at the Austrian Centre of Industrial Biotechnology (ACIB) and Graz University of Technology. His main interests deal with the combination of chemical and biocatalytical methods for the generation of chiral molecules, biopolymers, and pharmaceuticals.



Margit Winkler is currently a Senior Researcher at ACIB. She studied Technical Chemistry at Graz University of Technology. Her PhD was completed in 2005 at the Institute of Organic Chemistry at TUGraz. After 3 years as Scientific Assistant, she joined Prof. David O'Hagan at the University of St Andrews as Erwin-Schrödinger-fellow. Ever since, Margit's scientific focus has been the development of biocatalytic methods. She is using and develops enzymes and whole cell biocatalysts as tools for nitrile formation, hydrolysis and reduction as well as fluorination, hydroxylation, desaturation, oxidation and reduction, mainly of active pharmaceutical ingredients or building blocks thereof.

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10 References

- [1] Myriant Corporation, Myriant, ThyssenKrupp Uhde scale bio-succinic acid process. *Biomass Mag.* 2013, 7, 38.
- [2] Noweck, K., Grafarend, W., Fatty alcohols, *Ullmann's Encyclopedia of Industrial Chemistry*, Wiley-VCH, Weinheim 2006.
- [3] Yang, S.-T., Huang, H., Tay, A., Qin, W. et al., Extractive fermentation for the production of carboxylic acids, in: Yang, S.-T., (Ed.), *Bio-processing for Value-Added Products from Renewable Resources: New Technologies and Applications*, Elsevier B.V., Amsterdam 2007, pp. 421–446.
- [4] Cha, J. S., Recent developments in the reduction of carboxylic acids and their derivatives with metal hydrides. A review. *Org. Prep. Proced. Int. New J. Org. Synth.* 1989, 21, 451–477.
- [5] Cha, J. S., Thirty six years of research on the selective reduction and hydroboration. *Bull. Korean Chem. Soc.* 2012, 32, 1808–1846.
- [6] Harcken, C., Aliphatic and alicyclic aldehydes: Synthesis by reduction or by reduction followed by hydrolysis. *Sci. Synth.* 2007, 25, 65–135.
- [7] Tojo, G., Fernández, M., *Oxidation of Alcohols to Aldehydes and Ketones: A Guide to Current Common Practice*, Springer, New York, 2006.
- [8] Seydel-Penne, J., *Reductions by the Alumino- and Borohydrides in Organic Synthesis*, 2nd Edn., Wiley-VCH, Pittsburgh 1997.
- [9] Málek, J., Reductions by metal alkoxyaluminum hydrides. Part II. Carboxylic acids and derivatives, nitrogen compounds, and sulfur compounds. *Org. React.* 1988, 36, 249–590.
- [10] Dub, P. A., Ikariya, T., Catalytic reductive transformations of carboxylic and carbonic acid derivatives using molecular hydrogen. *ACS Catal.* 2012, 2, 1718–1741.
- [11] Addis, D., Das, S., Junge, K., Beller, M., Selective reduction of carboxylic acid derivatives by catalytic hydrosilylation. *Angew. Chem. Int. Ed. Engl.* 2011, 50, 6004–6011.
- [12] Hollmann, F., Arends, I. W. C. E., Holtmann, D., Enzymatic reductions for the chemist. *Green Chem.* 2011, 13, 2285–2314.
- [13] Farmer, V. C., Henderson, M. E. K., Russell, J. D., Reduction of certain aromatic acids to aldehydes and alcohols by *Polystictus versicolor*. *Biochim. Biophys. Acta* 1959, 35, 201–211.
- [14] Shimazono, H., Nord, F. F., Transformations of anisic acid and methylanisate by the mold *Polystictus versicolor*. *Arch. Biochem. Biophys.* 1959, 87, 140–143.
- [15] Arfmann, H.-A., Abraham, W.-R., Microbial reduction of aromatic carboxylic acids. *Z. Naturforsch. C J. Biosci.* 1993, 48, 52–57.
- [16] Nishida, A., Fukuzumi, T., Formation of coniferyl alcohol from ferulic acid by the white-rot fungus *Trametes*. *Phytochemistry* 1978, 17, 417–419.
- [17] Ishikawa, H., Schubert, W. J., Nord, F. F., Investigations on lignins and lignification XXVIII. The degradation by *Polyporus versicolor* and *Fomes fomentarius* of aromatic compounds structurally related to softwood lignin. *Arch. Biochem. Biophys.* 1963, 100, 140–149.
- [18] Gupta, J., Hamp, S., Buswell, J., Eriksson, K., Metabolism of trans-ferulic acid by the white-rot fungus *Sporotrichum pulverulentum*. *Arch. Microbiol.* 1981, 128, 349–354.
- [19] Falconnier, B., Lapierre, C., Lesage-Meessen, L., Yonnet, G. et al., Vanillin as a product of ferulic acid biotransformation by the white-rot fungus *Pycnoporus cinnabarinus* I-937: Identification of metabolic pathways. *J. Biotechnol.* 1994, 37, 123–132.
- [20] Lesage-Meessen, L., Delattre, M., Haon, M., Thibault, J. et al., A two-step bioconversion process for vanillin production from ferulic acid combining *Aspergillus niger* and *Pycnoporus cinnabarinus*. *J. Biotechnol.* 1996, 50, 107–113.
- [21] Thibault, J., Micard, V., Renard, C., Asther, M. et al., Fungal bioconversion of agricultural by-products to vanillin. *LWT – Food Sci. Technol.* 1998, 31, 530–536.
- [22] Mingjun, W., Pu, Z., Zhihao, S., Zhiqiang, Z., Microbial transformation of vanillic acid to vanillin by *Pycnoporus cinnabarinus* SW-0203. *Food Ferment. Ind.* 2004, 30, 43–47.
- [23] Zheng, L., Zheng, P., Sun, Z., Bai, Y. et al., Production of vanillin from waste residue of rice bran oil by *Aspergillus niger* and *Pycnoporus cinnabarinus*. *Bioresour. Technol.* 2007, 98, 1115–1119.
- [24] Vaithanomsat, P., Apivatanapiwat, W., Feasibility study on vanillin production from *Jatropha curcas* stem using steam explosion as a pretreatment. *Int. J. Chem. Biol. Eng.* 2009, 2, 211–214.
- [25] Sun, Z., *Vanillin strain prepared by microbial conversion and method thereof*. CN Patent 1770182 A, 2005.
- [26] Sun, Z., Zheng, P., Guo, X., Lin, G., et al., *Method for producing vanillic acid and vanillin from waste residue of rice bran oil by fermentation and biotransformation*. EP Patent 1734128 A1, 2006, 1–15.
- [27] Lesage-Meessen, L., Delattre, M., Haon, M., Asther, M., *Methods for bioconversion of ferulic acid to vanillic acid or vanillin and for the bioconversion of vanillic acid to vanillin using filamentous fungi*. US Patent 5866380, 1999.
- [28] Motedayen, N., Ismail, M. B. T., Nazarpour, F., Bioconversion of ferulic acid to vanillin by combined action of *Aspergillus niger* K8 and *Phanerochaete crysosporium* ATCC 24725. *Afr. J. Biotechnol.* 2013, 12, 6618–6624.
- [29] Dos Santos Barbosa, E., Perrone, D., do Amaral Vendramini, A. L., Ferreira Leite, S. G., Vanillin production by *Phanerochaete chrysosporium* grown on green coconut agro-industrial husk in solid state fermentation. *BioResources* 2008, 3, 1042–1050.
- [30] Lauritsen, F. R., Lundsgaard, A., A study of the bioconversion potential of the fungus *Bjerkandera adusta* with respect to a production of chlorinated aromatic compounds. *Enzyme Microb. Technol.* 1998, 22, 459–465.
- [31] Hage, A., Schoemaker, H. E., Field, J. A., Reduction of aryl acids by white-rot fungi for the biocatalytic production of aryl aldehydes and alcohols. *Appl. Microbiol. Biotechnol.* 1999, 55, 834–838.
- [32] Von Nussbaum, F., Spahlt, W., Steglich, W., Reduction of anthranilic acid and related amino acids in fruit bodies of *Hebeloma saccharolepis*. *Phytochemistry* 1997, 46, 261–264.
- [33] Bachman, D. M., Dragoon, B., John, S., Reduction of salicylate to saligenin by *Neurospora*. *Arch. Biochem. Biophys.* 1960, 91, 326.
- [34] Raman, T. S., Shanmugasundaram, E. R. B., Metabolism of some aromatic acids by *Aspergillus niger*. *J. Bacteriol.* 1962, 84, 1339–1340.
- [35] Kato, N., Konishi, H., Uda, K., Shimao, M., Sakazawa, C., Microbial reduction of benzoate to benzyl alcohol. *Agric. Biol. Chem.* 1988, 52, 1885–1886.
- [36] Kato, N., Konishi, H., Masuda, M., Joung, E.-H. et al., Reductive transformation of benzoate by *Nocardia asteroides* and *Hormoconis resinae*. *J. Ferment. Bioeng.* 1990, 69, 220–223.

- [37] Ma, L., Liu, X., Liang, J., Zhang, Z., Biotransformations of cinnamaldehyde, cinnamic acid and acetophenone with *Mucor*. *World J. Microbiol. Biotechnol.* 2011, **27**, 2133–2137.
- [38] Tsuda, Y., Kawai, K., Nakajima, S., Asymmetric reduction of 2-methyl-2-aryloxyacetic acids by *Glomerella cingulata*. *Agric. Biol. Chem.* 1984, **48**, 1373–1374.
- [39] Tsuda, Y., Kawai, K., Nakajima, S., Microbial reduction and resolution of herbicidal 2-alkyl-2-aryloxyacetic acids by *Gloeosporium olivaceum*. *Chem. Pharm. Bull.* 1985, **35**, 1955–1960.
- [40] Tsuda, Y., Kawai, K., Nakajima, S., Microbial reduction of 2-phenylpropionic acid, 2-benzoyloxypropionic acid and 2-(2-furfuryl)propionic acid. *Chem. Pharm. Bull.* 1985, **33**, 4657–4661.
- [41] Bock, G., Benda, I., Schreier, P., Reduction of cinnamaldehyde and unsaturated acids by *Botrytis cinerea*. *Z. Lebensm. -Unters. Forsch.* 1988, **186**, 33–35.
- [42] Li, T., Rosazza, J. P. N., Biocatalytic synthesis of vanillin. *Appl. Environ. Microbiol.* 2000, **66**, 684–687.
- [43] Villa, R., Molinari, F., Reduction of carbonylic and carboxylic groups by plant cell cultures. *J. Nat. Prod.* 2008, **71**, 693–696.
- [44] Gross, G. G., Bolkart, K. H., Zenk, M. H., Reduction of cinnamic acid to cinnamaldehyde and alcohol. *Biochem. Biophys. Res. Commun.* 1968, **32**, 173–178.
- [45] Gross, G. G., Zenk, M. H., Reduktion aromatischer Säuren zu Aldehyden und Alkoholen im zellfreien System. *Eur. J. Biochem.* 1969, **8**, 413–419.
- [46] Li, T., Rosazza, J. P. N., Purification, characterization, and properties of an aryl aldehyde oxidoreductase from *Nocardia* sp. strain NRRL 5646. *J. Bacteriol.* 1997, **179**, 3482–3487.
- [47] He, A., Li, T., Daniels, L., Fotheringham, I., Rosazza, J. P. N., *Nocardia* sp. carboxylic acid reductase: Cloning, expression, and characterization of a new aldehyde oxidoreductase family. *Appl. Environ. Microbiol.* 2004, **70**, 1874–1881.
- [48] Venkitasubramanian, P., Daniels, L., Rosazza, J. P. N., Reduction of carboxylic acids by *Nocardia* aldehyde oxidoreductase requires a phosphopantetheinylated enzyme. *J. Biol. Chem.* 2007, **282**, 478–485.
- [49] Sánchez, C., Du, L., Edwards, D., Toney, M., Shen, B., Cloning and characterization of a phosphopantetheinyl transferase from *Streptomyces verticillus* ATCC15003, the producer of the hybrid peptide-polyketide antitumor drug bleomycin. *Chem. Biol.* 2001, **8**, 725–738.
- [50] Venkitasubramanian, P., Daniels, L., Das, S., Lamm, A. S., Rosazza, J. P. N., Aldehyde oxidoreductase as a biocatalyst: Reductions of vanillic acid. *Enzyme Microb. Technol.* 2008, **42**, 130–137.
- [51] Hansen, E. H., Møller, B. L., Kock, G. R., Bünnér, C. M. et al., De novo biosynthesis of vanillin in fission yeast (*Schizosaccharomyces pombe*) and baker's yeast (*Saccharomyces cerevisiae*). *Appl. Environ. Microbiol.* 2009, **75**, 2765–2774.
- [52] Napora-Wijata, K., Robins, K., Osorio-Lozada, A., Winkler, M., Whole-cell carboxylate reduction for the synthesis of 3-hydroxytyrosol. *ChemCatChem* 2013, DOI: 10.1002/cctc.201300913.
- [53] Binder, T. P., *Enzymatic method of making aldehydes from fatty acids*. US Patent Application 2007/0281345, 2007.
- [54] Akhtar, M. K., Turner, N. J., Jones, P. R., Carboxylic acid reductase is a versatile enzyme for the conversion of fatty acids into fuels and chemical commodities. *Proc. Natl. Acad. Sci. USA* 2013, **110**, 87–92.
- [55] Simon, H., White, H., Lebertz, H., Thanos, I., Reduction of 2-enoates and alkanoates with carbon monoxide or formate, viologenes, and *Clostridium thermoaceticum* to saturated acids and unsaturated and saturated alcohols. *Angew. Chem. Int. Ed. Engl.* 1987, **26**, 785–787.
- [56] Fraisse, L., Simon, H., Observations on the reduction of non-activated carboxylates by *Clostridium formicoaceticum* with carbon monoxide or formate and the influence of various viologens. *Arch. Microbiol.* 1988, **150**, 381–386.
- [57] White, H., Feicht, R., Huber, C., Lottspeich, F., Simon, H., Purification and some properties of the tungsten-containing carboxylic acid reductase from *Clostridium formicoaceticum*. *Biol. Chem. Hoppe-Seyler* 1991, **372**, 999–1005.
- [58] White, H., Strobl, G., Feicht, R., Simon, H., Carboxylic acid reductase: A new tungsten enzyme catalyses the reduction of non-activated carboxylic acids to aldehydes. *Eur. J. Biochem.* 1989, **96**, 89–96.
- [59] Huber, C., Skopan, H., Feicht, R., White, H., Simon, H., Pterin cofactor, substrate specificity, and observations on the kinetics of the reversible tungsten-containing aldehyde oxidoreductase from *Clostridium thermoaceticum*. *Arch. Microbiol.* 1995, **164**, 110–118.
- [60] White, H., Huber, C., Feicht, R., Simon, H., On a reversible molybdenum-containing aldehyde oxidoreductase from *Clostridium formicoaceticum*. *Arch. Microbiol.* 1993, **159**, 244–249.
- [61] Perez, J. M., Richter, H., Loftus, S. E., Angenent, L. T., Biocatalytic reduction of short-chain carboxylic acids into their corresponding alcohols with syngas fermentation. *Biotechnol. Bioeng.* 2013, **110**, 1066–1077.
- [62] Richter, H., Qureshi, N., Heger, S., Dien, B. et al., Prolonged conversion of *n*-butyrate to *n*-butanol with *Clostridium saccharoperbutylacetonicum* in a two-stage continuous culture with in-situ product removal. *Biotechnol. Bioeng.* 2012, **109**, 913–921.
- [63] Ježo, I., Zemek, J., Enzymatische Reduktion einiger aromatischer Carboxysäuren. *Chem. Pap.* 1986, **40**, 279–281.
- [64] Gentner, B. R., Townsend, G. T., Blattmann, B. O., Reduction of 3-chlorobenzoate, 3-bromobenzoate, and benzoate to corresponding alcohols by *Desulfomicrobium escambienense*, isolated from a 3-chlorobenzoate-dechlorinating coculture. *Appl. Environ. Microbiol.* 1997, **63**, 4698–4703.
- [65] Bock, M., Kneifel, H., Schöberth, S. M., Sahm, H., Reduction of halogenated derivatives of benzoic acid to the corresponding alcohols by *Desulfovibrio vulgaris* PY1. *Acta Biotechnol.* 2000, **20**, 189–201.
- [66] Agler, M. T., Wrenn, B. A., Zinder, S. H., Angenent, L. T., Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. *Trends Biotechnol.* 2011, **29**, 70–78.
- [67] Marshall, C. W., Labelle, E. V., May, H. D., Production of fuels and chemicals from waste by microbiomes. *Curr. Opin. Biotechnol.* 2013, **24**, 391–397.
- [68] Steinbusch, K. J. J., Hamelers, H. V. M., Shaap, J. D., Kampman, C., Buisman, C. J. N., Alcohol production through volatile fatty acids reduction with hydrogen as electron donor by mixed cultures. *Water Res.* 2008, **42**, 4059–4066.
- [69] Steinbusch, K. J. J., Hamelers, H. V. M., Shaap, J. D., Kampman, C., Buisman, C. J. N., Bioelectrochemical ethanol production through mediated acetate reduction by mixed cultures. *Environ. Sci. Technol.* 2010, **44**, 513–517.
- [70] Sharma, M., Aryal, N., Sarma, P. M., Vanbroekhoven, K. et al., Bioelectrocatalyzed reduction of acetic and butyric acids via direct electron transfer using a mixed culture of sulfate-reducers drives electrosynthesis of alcohols and acetone. *Chem. Commun.* 1999, **49**, 6495–6497.
- [71] Van den Ban, E. C. D., Willemen, H. M., Wassink, H., Haaker, H., Laane, C., Bioreductions by *Pyrococcus furiosus* at elevated temperatures. *Prog. Biotechnol.* 1998, **15**, 619–624.
- [72] Van den Ban, E. C. D., Willemen, H. M., Wassink, H., Laane, C., Haaker, H., Bioreduction of carboxylic acids by *Pyrococcus furiosus* in batch cultures. *Enzyme Microb. Technol.* 1999, **25**, 251–257.
- [73] Ni, Y., Hagedoorn, P., Xu, J., Arends, I. W. C. E., Hollmann, F., A biocatalytic hydrogenation of carboxylic acids. *Chem. Commun.* 2012, **48**, 12056–12058.
- [74] Ni, Y., Hagedoorn, P.-L., Xu, J.-H., Arends, I. W. C. E., Hollmann, F., *Pyrococcus furiosus*-mediated reduction of conjugated carboxylic acids: Towards using syngas as reductant. *J. Mol. Catal. B Enzym.* 2013, DOI: 10.1016/j.molcatb.2013.09.006.

- [75] Mukund, S., Adams, M. W. W., Characterization of a tungsten-iron-sulfur protein exhibiting spectroscopic and redox properties from the hyperthermophilic archaeabacterium *Pyrococcus furiosus*. *J. Biol. Chem.* 1990, **265**, 11508–11516.
- [76] Mukund, S., Adams, M. W. W., The novel tungsten-iron-sulfur protein of the hyperthermophilic archaeabacterium, *Pyrococcus furiosus*, is an aldehyde ferredoxin oxidoreductase. Evidence for its participation in a unique glycolytic pathway. *J. Biol. Chem.* 1991, **266**, 14208–14216.
- [77] Bevers, L. E., Hagedoorn, P.-L., Hagen, W. R., The bioinorganic chemistry of tungsten. *Coord. Chem. Rev.* 2009, **293**, 269–290.
- [78] Heider, J., Ma, K., Adams, M. W. W., Purification, characterization, and metabolic function of tungsten-containing aldehyde ferredoxin oxidoreductase from the hyperthermophilic and proteolytic archaeon *Thermococcus* strain ES-1. *J. Bacteriol.* 1995, **177**, 4757–4764.
- [79] Meighen, E. A., Bacterial bioluminescence: organization, regulation and application of the lux genes. *FASEB J.* 1993, **7**, 1016–1022.
- [80] Liu, R., Zhu, F., Lu, L., Fu, A. et al, Metabolic engineering of fatty acyl-ACP reductase-dependent pathway to improve fatty alcohol production in *Escherichia coli*. *Metab. Eng.* 2014, **22**, 10–21.
- [81] Suvarna, K., Seah, L., Bhattacharjee, V., Bhattacharjee, J. K., Molecular analysis of the LYS2 gene of *Candida albicans*: Homology to peptide antibiotic synthetases and the regulation of the alpha-aminoacid reductase. *Curr. Gen.* 1998, **33**, 268–275.
- [82] Guo, S., Bhattacharjee, J. K., Posttranslational activation, site-directed mutation and phylogenetic analyses of the lysine biosynthesis enzymes α-aminoacid reductase Lys1p (AAR) and the phosphopantetheinyl transferase Lys7p (PPTase) from *Schizosaccharomyces pombe*. *Yeast* 2004, **21**, 1279–1288.
- [83] Suzuki, H., Ohnishi, Y., Horinouchi, S., GriC and GriD constitute a carboxylic acid reductase involved in grixazone biosynthesis in *Streptomyces griseus*. *J. Antibiot.* 2007, **60**, 380–387.
- [84] Koketsu, K., Watanabe, K., Suda, H., Oguri, H., Oikawa, H., Enzymatic reconstruction of the saframycin core scaffold defines dual Pictet-Spengler mechanisms. *Nat. Chem. Biol.* 2010, **6**, 408–410.
- [85] Doan, T. T. P., Carlsson, A. S., Hamberg, M., Bülow, L. et al., Functional expression of five *Arabidopsis* fatty acyl-CoA reductase genes in *Escherichia coli*. *J. Plant Physiol.* 2009, **166**, 787–796.
- [86] Teerawanichpan, P., Robertson, A. J., Qiu, X., A fatty acyl-CoA reductase highly expressed in the head of honey bee (*Apis mellifera*) involves biosynthesis of a wide range of aliphatic fatty alcohols. *Insect Biochem. Mol. Biol.* 2010, **40**, 641–649.
- [87] Teerawanichpan, P., Qiu, X., Fatty acyl-CoA reductase and wax synthase from *Euglena gracilis* in the biosynthesis of medium-chain wax esters. *Lipids* 2010, **45**, 263–273.



Special Issue: Industrial Biotechnology. This Special Issue, edited by Jarka Glassey and Marcel Ottens, contains articles from the 2nd European Conference on Applied Biotechnology (ECAB2) in The Hague, The Netherlands, April 2013. The cover image shows a tubular photobioreactor for cultivation of microalgae. It is the most common large-scale reactor type for production of high and medium algae products. The picture is provided by Clemens Posten (KIT, Karlsruhe, Germany), co-author of the Review on "Biorefinery of microalgae – opportunities and constraints for different production scenarios".
<http://dx.doi.org/10.1002/biot.201300142>

Biotechnology Journal – list of articles published in the June 2014 issue.

Editorial: Industrial biotechnology – Technologies and methods for rapid process development

Jarka Glassey and Marcel Ottens

<http://dx.doi.org/10.1002/biot.201400304>

Perspective

Hybrid modeling for quality by design and PAT – benefits and challenges of applications in biopharmaceutical industry

Moritz von Stosch, Steven Davy, Kjell Francois, Vytautas Galvanauskas, Jan-Martijn Hamelink, Andreas Luebbert, Martin Mayer, Rui Oliveira, Ronan O'Kennedy, Paul Rice and Jarka Glassey

<http://dx.doi.org/10.1002/biot.201300385>

Perspective

Challenges in industrial fermentation technology research

Luca Riccardo Formenti, Anders Nørregaard, Andrijana Bolic, Daniela Quintanilla Hernandez, Timo Hagemann, Anna-Lena Heins, Hilde Larsson, Lisa Mears, Miguel Mauricio-Iglesias, Ulrich Kriühne and Krist V. Gernaey

<http://dx.doi.org/10.1002/biot.201300236>

Review

Biorefinery of microalgae – opportunities and constraints for different production scenarios

Ioanna Hariskos and Clemens Posten

<http://dx.doi.org/10.1002/biot.201300142>

Research Article

Assessing the environmental sustainability of ethanol from integrated biorefineries

Temitope Falano, Harish K. Jeswani and Adisa Azapagic

<http://dx.doi.org/10.1002/biot.201300246>

Research Article

Economics of recombinant antibody production processes at various scales: Industry-standard compared to continuous precipitation

Nikolaus Hammerschmidt, Anne Tscheliessnig, Ralf Sommer, Bernhard Helk and Alois Jungbauer

<http://dx.doi.org/10.1002/biot.201300480>

Research Article

iOD907, the first genome-scale metabolic model for the milk yeast *Kluyveromyces lactis*

Oscar Dias, Rui Pereira, Andreas K. Gombert, Eugénio C. Ferreira and Isabel Rocha

<http://dx.doi.org/10.1002/biot.201300242>

Research Article

Aerobic expression of *Vitreoscilla hemoglobin* efficiently reduces overflow metabolism in *Escherichia coli*

Tania E. Pablos, Juan Carlos Sigala, Sylvie Le Borgne and Alvaro R. Lara

<http://dx.doi.org/10.1002/biot.201300388>

Technical Report

Prediction of IgG1 aggregation in solution

Frida Ojala, Marcus Degerman, Thomas Budde Hansen, Ernst Broberg Hansen and Bernt Nilsson

<http://dx.doi.org/10.1002/biot.201400018>

Technical Report

Robust, microfabricated culture devices with improved control over the soluble microenvironment for the culture of embryonic stem cells

Rhys J. Macown, Farlan S. Veraitch and Nicolas Szita

<http://dx.doi.org/10.1002/biot.201300245>

Technical Report

Enzymatic hydration activity assessed by selective spectrophotometric detection of alcohols: A novel screening assay using oleate hydratase as a model enzyme

Aida Hiseni, Rosario Medici, Isabel W. C. E. Arends and Linda G. Otten

<http://dx.doi.org/10.1002/biot.201300412>

Regular Articles

Review

Biocatalytic reduction of carboxylic acids

Kamila Napora-Wijata, Gernot A. Strohmeier and Margit Winkler

<http://dx.doi.org/10.1002/biot.201400012>

Research Article

In vivo and in vitro activity of an immunoglobulin Fc fragment (Fcab) with engineered Her-2/neu binding sites

Max Woisetschläger, Bernhard Antes, Radha Borrowdale, Susanne Wiederkum, Manuela Kainer, Herta Steinkellner, Gordana Wozniak-Knopp, Kevin Moulder, Florian Rücker and Geert C. Mudde

<http://dx.doi.org/10.1002/biot.201300387>

Research Article

Microscopic monitoring provides information on structure and properties during biocatalyst immobilization

Sarka Bidmanova, Eva Hrdlickova, Josef Jaros, Ladislav Ilkovics, Ales Hampl, Jiri Damborsky and Zbynek Prokop

<http://dx.doi.org/10.1002/biot.201300049>