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MINIREVIEW

Microbial Engineering for Aldehyde Synthesis

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Aldehydes are a class of chemicals with many industrial uses. Several aldehydes are responsible for flavors and fragrances present in plants, but aldehydes are not known to accumulate in most natural microorganisms. In many cases, microbial production of aldehydes presents an attractive alternative to extraction from plants or chemical synthesis. During the past 2 decades, a variety of aldehyde biosynthetic enzymes have undergone detailed characterization. Although metabolic pathways that result in alcohol synthesis via aldehyde intermediates were long known, only recent investigations in model microbes such as *Escherichia coli* have succeeded in minimizing the rapid endogenous conversion of aldehydes into their corresponding alcohols. Such efforts have provided a foundation for microbial aldehyde synthesis and broader utilization of aldehydes as intermediates for other synthetically challenging biochemical classes. However, aldehyde toxicity imposes a practical limit on achievable aldehyde titers and remains an issue of academic and commercial interest. In this minireview, we summarize published efforts of microbial engineering for aldehyde synthesis, with an emphasis on *de novo* synthesis, engineered aldehyde accumulation in *E. coli*, and the challenge of aldehyde toxicity.

The word "aldehyde" was coined in the early 19th century by Justin von Liebig, who formed a contraction using the Latin words "alcohol dehydrogenatus," or "alcohol deprived of hydrogen" (1). Aldehydes have a variety of industrial uses, but they are perhaps most familiar for their effects on two of the mammalian senses: olfaction and gustation. Numerous aldehyde odorants are known to bind to G-protein-coupled receptors, triggering reaction cascades that ultimately result in mammalian perception (2–5). At dilute concentrations, fatty aldehydes such as hexanal, octanal, decanal, and dodecanal offer apple, citrus, orange peel, and violet scents, respectively (6). Aromatic aldehydes, such as benzaldehyde, anisaldehyde, vanillin, and cinnamaldehyde, are responsible for the natural fragrances of almond, sweet blossom, vanilla, and cinnamon, respectively (6, 7). Notable terpenoid aldehydes include citral, which provides lemon scent (6), and safranal, which is one of the primary molecules responsible for saffron aroma (8). Aldehydes play a role in other animal phyla as well. Certain aldehydes, such as trans-2-hexenal, phenylacetaldehyde, and nonanal, evoke responses in insects by serving as pheromones or attractants (9–11). The high reactivity of the carbonyl group of aldehydes enables many industrial uses beyond flavors and fragrances, such as precursors to pharmaceuticals (12–15). However, the high reactivity of aldehydes also contributes to their increased toxicity in microorganisms. Given the high-value applications and large markets for several aldehydes, commercial focus on microbial aldehyde synthesis has surged in recent years (16). This minireview summarizes published efforts of microbial engineering for aldehyde synthesis, with an emphasis on *de novo* aldehyde synthesis, engineered aldehyde accumulation in *Escherichia coli*, and the challenge of aldehyde toxicity.

ENGINEERING ALDEHYDE BIOSYNTHETIC REACTIONS AND PATHWAYS

Because most microbes do not naturally accumulate aldehydes, microbial production of these molecules from simple carbon sources requires at least two parallel approaches: pathway construction for product generation and strain engineering for product accumulation. A starting point for pathway construction is consideration of enzymatic reactions that can produce desired aldehydes from cellular metabolites. Carboxylic acids are found throughout cellular metabolism, and many can be converted to aldehydes with the aid of a single enzyme. Prior to the detailed characterization and cloning of enzymes capable of broadly catalyzing aldehyde formation, various natural organisms ranging from actinomycetes to white rot fungi were tested for the innate ability to convert carboxylic acids into their corresponding aldehydes or alcohols (17–21). A significant advance occurred roughly 1 decade ago, when a carboxylic acid reductase (Car_{Ni}) from *Nocardia iowensis* was cloned into *Escherichia coli* and shown to be active on several aromatic carboxylic acids *in vitro* (22). Later publications from Rosazza and colleagues demonstrated that Car_{Ni} requires one-time activation by a phosphopantetheinyl transferase and that Car_{Ni} has activity *in vitro* on a broader range of substrates that includes several citric acid cycle dicarboxylic acids (23, 24). Motivated by the activity of Car_{Ni} on diverse carboxylic acid substrates, we investigated its activity on straight-chain and branched-chain aliphatic acids ranging from C_2 to C_8 (25). A homolog of Car_{Ni} from *Mycobacterium marinum* was also demonstrated to have activity on straight-chain aliphatic acids ranging from C_6 to C_{18} (26). A recent review describes a larger number of carboxylic acid reductases that could be harnessed for biosynthesis of a variety of aldehydes (27). The general stoichiometry for reactions catalyzed by carboxylic acid reductases is as follows (where "e^-" represents a reducing equivalent):

$$\text{R-COOH} + e^- + \text{ATP} \rightarrow \text{R-CHO} + \text{AMP} + \text{PP}_i \quad (1)$$

Aliphatic aldehydes across a broad range of carbon lengths can also be formed by using fermentative aldehyde reductases or by

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using enzymes that act on activated forms of carboxylic acids (acyl-coenzyme A [CoA] or acyl-ACP). During anaerobic cultivation of *E. coli*, conversion of acetyl-CoA to acetaldehyde is catalyzed by a CoA-dependent acetaldehyde dehydrogenase (also known as acetaldehyde CoA dehydrogenase) (28). However, the same protein, encoded by *adhE*, has a second catalytic site that converts acetaldehyde into ethanol (29). In solvent-producing clostridial strains, acetaldehyde and butyraldehyde can be produced by CoA-acylating aldehyde dehydrogenases that are found as individual enzymes or as bifunctional enzymes (30–33). The conversion of acyl-CoA to aldehyde is as follows (for acyl-ACP substrates instead of acyl-CoA substrates, replace "S-CoA" and "CoASH" with "ACP"):

$$\text{R-CO-S-CoA} + e^- \rightarrow \text{R-CHO} + \text{CoASH} \quad (2)$$

Synthesis of longer carbon-chain aliphatic aldehydes from acyl-ACP precursors can occur using enzymes from luminescent bacteria. In these bacteria, the multienzyme fatty acid reductase complex consisting of *luxCDE* is used to produce aldehydes that are immediate substrates for the light emission reaction (34). Note that the aldehyde biosynthetic reactions discussed so far use similar chemistries that primarily differ in the source of reducing equivalents and whether the carboxylic acid molecule or the reductase enzyme is activated first. In either case, activation requires the conversion of ATP to AMP and pyrophosphate and occurs because the energetics of converting a carboxylic acid to an aldehyde are ordinarily unfavorable.

Another set of nonoxidative aldehyde biosynthetic routes utilizes decarboxylation of 2-keto acid substrates. In these cases, no ATP is required because the irreversibility of CO_2 formation provides the driving force for aldehyde formation. However, one carbon atom is lost per molecule of 2-keto acid substrate, which reduces the theoretical maximum yield. Two well-known enzymes in this category are pyruvate decarboxylase (PDC) and 2-ketoisovalerate decarboxylase (KivD). The native role of PDCs is to convert pyruvate to acetaldehyde, but their promiscuity and capability of catalyzing carboligation side reactions have led to their use in synthesis of chiral carboligation products (12). KivD is also promiscuous and has been utilized for synthesis of numerous nonnatural alcohols derived from amino acid intermediates (35). The 2-keto acid decarboxylation reaction is as follows:

$$\text{R-CO-COOH} \rightarrow \text{R-CHO} + CO_2 \quad (3)$$

Oxidative reactions can also be used for aldehyde synthesis, starting from either carboxylic acid substrates or primary alcohol substrates. C_n fatty acids can be converted to C_{n-1} fatty aldehydes, as was shown using *E. coli* resting cells that expressed an α-dioxygenase from *Oryza sativa* (rice) (36). In this case, spontaneous decarboxylation of a C_n hydroperoxy fatty acid intermediate provides a driving force for aldehyde generation. The dioxygenase-catalyzed reaction is as follows:

$$\text{R-CH}_2\text{-COOH} + O_2 \rightarrow \text{R-CHO} + CO_2 + H_2O \quad (4)$$

In addition, aldehydes can be obtained by enzymatic oxidation of primary alcohols (37–40). From a *de novo* aldehyde synthesis perspective, these reactions are less relevant given that alcohols are typically produced via aldehyde intermediates. However, biocatalytic conversion of primary alcohols to aldehydes may provide an array of new opportunities for alcohols as starting materials and is revisited later in this review. Oxidation of alcohols to aldehydes generates a reducing equivalent as follows:

$$\text{R-CH}_2\text{-OH} \rightarrow \text{R-CHO} + e^- \quad (5)$$

Natural and engineered pathways could be used to produce useful aldehydes from simple carbon sources via their corresponding carboxylic acids. Pathway selection leading to the relevant carboxylic acid precursor depends on the category of target aldehyde. Figure 1 illustrates known aromatic and aliphatic acid biosynthesis pathways that can be engineered to result in several familiar flavors and fragrances. In the case of vanillin, which has the largest annual market volume of any flavor compound, previous reports have described engineered heterologous pathways that use the natural aromatic amino acid precursor 3-dehydroshikimate as a branch-point metabolite for the heterologous reactions (41–43). Li and Frost constructed a system to produce vanillin from glucose that used an engineered strain of *E. coli* to produce vanillate from glucose, followed by extraction and reduction of vanillate to vanillin *in vitro* using purified carboxylic acid reductase from *Neurospora crassa* (41). *De novo* biosynthesis of vanillin and vanillin-β-D-glucoside was first demonstrated in both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* and has since been optimized using flux balance analysis (42, 44, 45). In initial reports, titers of *de novo* vanillin-β-D-glucoside were roughly 50 mg/liter in batch flask cultures (42) and 500 mg/liter in 1.5-liter continuous cultures (44). The company Evolva has improved and commercialized this process (16).

Among flavor compounds, benzaldehyde has the second largest annual market volume after vanillin (46). Aromatic amino acid biosynthesis could also be used to engineer a microbial pathway to benzaldehyde, potentially from phenylalanine as the starting endogenous metabolite. Formation of benzaldehyde was reported after phenylalanine addition to a cell extract of *Lactobacillus plantarum* (47). In plants, benzaldehyde is derived from phenylalanine, potentially from β-oxidative and non-β-oxidative pathways (48). Recent work has uncovered key steps in the β-oxidative pathway that can lead to synthesis of benzoate, which could serve as the precursor to benzaldehyde in an engineered microbial pathway (49).

Aliphatic aldehydes can be obtained using pathways that result in free fatty acids (FFAs). Although microbial FFAs have been produced for decades, recent work has demonstrated the potential for obtaining advanced fuels or valuable chemicals as derivatives of FFAs (50–53). Based on the broad substrate range and known activities of carboxylic acid reductases, their addition to these pathways can result in production of C_4 to C_{18} aliphatic aldehydes (25, 26). Microbial synthesis of other valuable aldehyde classes, such as terpenoid aldehydes, could potentially occur in *E. coli* using variations of previously engineered terpenoid pathways (54).

As mentioned earlier, commercial entities have actively pursued aldehyde biosynthesis routes using engineered microbes. Table 1 contains an overview of relevant published aldehyde biosynthesis patent applications during the past 30 years. These patents were grouped into three types of dominant routes of aldehyde biosynthesis. Although the third category is the most pertinent to the topic of this review, the other two categories of processes were included to provide context and perspective into chronological trends. For example, during the 1980s and 1990s, industry patents on biotransformation processes featured either isolated microbes or fruit homogenates. Commercial processes featuring fully *de novo* aldehyde synthesis using engineered microbes appear to have

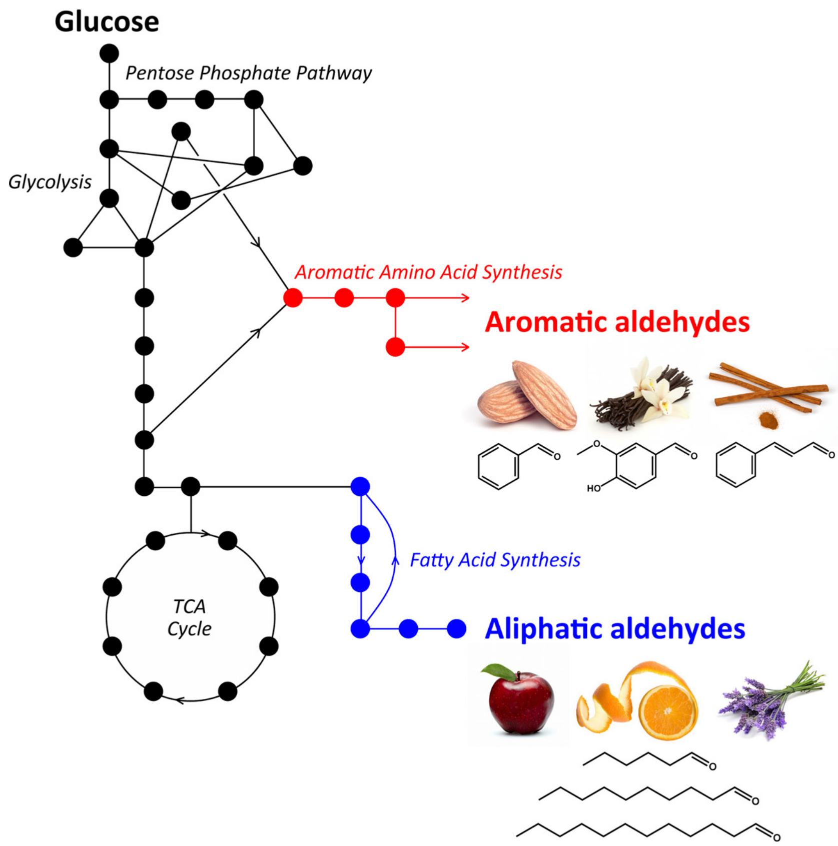

FIG 1 Overview of natural metabolic pathways that can be harnessed for the conversion of glucose to valuable aromatic and aliphatic aldehydes through carboxylic acid intermediates based on *E. coli* metabolism. Aldehydes can also be obtained from the 2-keto acid pathway (35, 55), terpenoid pathways (54), and other pathways. TCA, tricarboxylic acid.

emerged only within the last decade. Of course, an overview of patent literature does not account for industrial advances that were retained as trade secrets.

MINIMIZING ENDOGENOUS CONVERSION OF ALDEHYDES TO ALCOHOLS

Despite known routes to a variety of aldehydes, microbial aldehyde production is hindered by the rapid endogenous conversion of nearly all aldehydes to their corresponding alcohols. For example, when expression of recombinant Car_{Ni} was first reported in *E. coli*, aromatic acids supplied to culture media were rapidly converted into aromatic alcohols (22). Even in *E. coli*, the genetically best-understood organism, numerous uncharacterized genes were thought to contribute to this activity. To our knowledge, explanations of how to significantly reduce endogenous conversion for any given aldehyde in *E. coli* became present in the public domain only very recently. It is worth highlighting here that, although oxidation of an aldehyde to a carboxylic acid is thermodynamically more favorable than reduction of a carboxylic acid to an aldehyde, endogenous aldehyde oxidation does not appear to be significant for most aldehydes of interest in model microbes. On the other hand, endogenous aldehyde reduction has been thoroughly documented in the literature and is the focus of this review.

In 2012, Rodriguez and Atsumi reported accumulation of isobutyraldehyde in *E. coli* by sequentially deleting eight genes (*yqhD*, *adhP*, *eutG*, *yiaY*, *yjgB* [now *ahr*], *betA*, *fucO*, and *eutE*) encoding putative isobutyraldehyde reductases (55). When individually overexpressed, five of these genes displayed activity toward isobutyraldehyde. The engineered deletion strain increased isobutyraldehyde production from 0.14 g/liter/optical density at 600 nm (OD_{600}) to 1.5 g/liter/OD_{600} and decreased isobutanol production from 1.5 g/liter/OD_{600} to 0.4 g/liter/OD_{600}. Although isobutanol formation still occurred, that study suggested that the number of gene deletions required to mitigate conversion of a particular aldehyde may be a manageable quantity.

We became interested in determining whether gene deletions could enable accumulation of aromatic aldehydes and believed that fewer gene deletions might be required for accumulation under aerobic conditions. After deletion of six genes that encode enzymes with confirmed activity on benzaldehyde *in vitro* (*dkgA*, *dkgB*, *yeaE*, *yahK*, *ahr*, and *yqhD*), the engineered *E. coli* strain accumulated benzaldehyde and vanillin with minimal alcohol formation and was thus dubbed "RARE" for displaying reduced aromatic aldehyde reduction (Addgene catalog no. 61440) (43). Each targeted gene was capable of causing reduction of benzaldehyde and vanillin *in vivo* when individually overexpressed in the RARE background. However, the use of deletion subset strains and quantitative reverse transcription-PCR (qRT-PCR) revealed that deletions of *dkgB* and *yeaE* did not contribute to aldehyde accumulation under the conditions tested due to low native expression of these genes (43).

Soon after aromatic aldehyde accumulation was reported, Rodriguez and Atsumi reported the construction of an *E. coli* strain that minimally converted exogenously supplied aliphatic aldehydes ranging from C_2 to C_{12} to their corresponding alcohols (56). Their study examined 44 candidate aldehyde reductases *in vivo* by overexpressing candidates using the previously reported isobutraldehyde-accumulating strain (55). However, overexpression of genes encoding aldehyde reductases has been shown to lead to false positives when such genes are minimally expressed under relevant conditions (43). Rodriguez and Atsumi noted that fewer than the 13 genes deleted in their final strain (*yqhD*, *adhP*, *eutG*, *yiaY*, *ahr*, *betA*, *fucO*, *eutE*, *yahK*, *dkgA*, *gldA*, *ybbO*, and *yghA*) may be sufficient to create useful strains devoted to a specific set of aldehyde products (56). Given that the consequential gene deletions in the RARE strain form a subset of the genes deleted by Rodriguez and Atsumi, both strains are likely capable of accumulating most aromatic and aliphatic aldehydes of interest under aerobic conditions at the shake flask scale. Under other conditions, such as high-cell-density industrial fermentations that commonly feature anaerobic zones, it may be better to err on the side of inclusion of more deletions as long as cell health and stability are not significantly perturbed. Together, these studies should aid efforts to engineer aldehyde accumulation in other microbes.

ENHANCING BIOCONVERSION OF ALDEHYDES TO OTHER CHEMICAL CLASSES

Microbial aldehyde accumulation enables biosynthesis of several previously problematic compounds that can be derived enzymatically from aldehyde intermediates (Fig. 2). In our report on aromatic aldehyde accumulation (43), we demonstrated this potential by using the RARE strain to produce L-phenylacetylcarbinol (L-PAC), a chiral precursor to the pharmaceutical ephedrine (12–15). Although whole-cell catalysts have been used for L-PAC synthesis for a long time, significant benzyl alcohol byproduct formation occurs from their use, resulting in low yields (12). Cultures of the RARE strain expressing a recombinant mutant PDC were able to produce L-PAC using exogenously supplied benzaldehyde and metabolized pyruvate with minimal benzyl alcohol formation. Under the conditions tested, the use of wild-type *E. coli* expressing the same PDC produced no detectable L-PAC (43). In addition to PDC, other enzymes capable of catalyzing chiral carboligations of aldehyde substrates have been discussed (57).

A similar challenge of limiting unwanted flux from aldehyde intermediates to alcohol byproducts has been encountered in the context of alkane production. The final step to alkane biosynthesis features the conversion of a C_n aldehyde to a C_{n-1} alkane catalyzed by an aldehyde decarbonylase or aldehyde deformylating oxygenase (26, 58–62). Although the problem of alcohol byproduct formation has been described extensively, very few studies of alkane biosynthesis have used strains engineered with deletions of aldehyde reductases. Rodriguez and Atsumi discussed the relevance of their strain for alkane synthesis but did not demonstrate alkane production in their study (56). Production of propane was recently reported by Kallio and colleagues using engineered *E. coli* that displayed decreased endogenous conversion of butyraldehyde to butanol due to deletions of *ahr* and *yqhD* (63).

In addition to chiral carboligations and decarbonylations, aldehyde substrates can participate in numerous other enzyme-catalyzed reactions (Fig. 2), for example, transamination to form primary amines (64, 65), hydrocyanation to form chiral cyanohydrins (66), Henry reactions to form nitroalcohols (67), Baeyer-Villager oxidation to form esters (68), and Mannich reactions to form β-amino-carbonyl compounds (69, 70). Some of the aforementioned reactions have already been demonstrated to be functional in a cellular context using resting *E. coli* cells (66, 71). Microbial aldehyde accumulation enables potential synthesis of these compounds using metabolically active cells that can supply and regenerate expensive cofactors. Synthesis of some of these products may also be achieved using glucose or other simple sugars as the sole carbon source. In addition, biocatalytic oxidation of exogenously supplied alcohols (37–40, 64, 72) would be more effective in the absence of aldehyde reduction. In theory, any of the classes of aldehyde-derived compounds enabled in the absence of aldehyde reduction could also be obtained directly from the corresponding primary alcohols using a single engineered microbe.

ADDRESSING ALDEHYDE TOXICITY

Now that published reports have elucidated aldehyde accumulation in *E. coli* under laboratory-scale conditions, the next impediment to engineering microbial aldehyde synthesis is aldehyde toxicity. Observable toxicity is manifested by inhibition of microbial growth in the presence of aldehydes (43, 73), but morphological changes have also been reported (73). In most cases, the extent of toxicity seems to depend on the aldehyde but may also depend on the choice of microorganism. Cinnamaldehyde, for example, is known to be a potent antimicrobial (74). In the case of vanillin, Zaldivar et al. found that 1.5 g/liter of vanillin completely inhibited growth of the *E. coli* strains examined (73). The same study investigated the effect of exposing *E. coli* to several representative aromatic aldehyde products of hemicellulose hydrolysis and found that toxicity was directly related to the hydrophobicity of the aldehyde. The relationship with hydrophobicity suggested that a hydrophobic target, such as the cell membrane, may be involved. However, none of these aldehydes caused sufficient membrane damage to allow the leakage of intracellular magnesium (73). Another study investigated the toxicity of four aldehydes (furfural, 5-hydroxymethylfurfural, vanillin, and syringaldehyde) to *Candida tropicalis* and found that vanillin was the most toxic, followed by syringaldehyde, furfural, and 5-hydroxymethylfurfural (75). The influence of the structural elements of vanillin and related compounds on antifungal activity has also been examined, and differences in antifungal activity were found (76). However, when the effect of five aldehydes on the growth of the oleaginous yeast *Trichosporon fermentans* was investigated, no relationship was found between the hydrophobicity and toxicity of the aldehyde (77).

The *E. coli* strains investigated by Zaldivar et al. were not engineered to have minimal aldehyde reductase activity, and later studies from the same group suggested that growth inhibition may be caused by NADPH consumption resulting from aldehyde reduction (78, 79). Two genes (*dkgA* and *yqhD*) were found to be silenced in an evolved furfural-resistant strain. Expression of these genes, which encode enzymes with low K_m values for NADPH, decreased furfural tolerance (78). In a separate investigation, transcriptome data were analyzed before and after exposure to furfural. Several lines of evidence suggested that cysteine and methionine biosynthesis was upregulated in order to combat a limitation in sulfur assimilation due to NADPH depletion (79). Although NADPH consumption may contribute to toxicity, our experience with aldehyde accumulation suggests that aromatic al-

TABLE 1 Relevant published aldehyde biosynthesis patent applications

Dominant aldehyde biosynthesis route	Applicant	Publication date	Publication no.	Patent name	Relevant claim(s)[a]	Grant (G) or application (A)
Biotransformation using homogenates or natural microorganisms	Takasago Perfumery	6 Sep 1988	US 4769243 A	Method for preparing green aroma compounds	Use of ground soybeans to convert unsaturated fatty acids to aliphatic aldehydes and alcohols	G
	General Foods Corporation	21 February 1989	US 4806379 A	Process for producing a green leaf essence	Use of strawberry homogenate to convert linolenic acid to cis-3-hexanal and related aldehydes	G
	BASF	17 October 1989	US 4874701 A	Preparation of coniferylaldehyde by a microorganism	Use of *Arthrobacter globiformis* DSM 3597 to convert n-eugenol to coniferylaldehyde	G
	Haarmann & Reimer GmbH	21 May 1991	US 5017388	Process for the preparation of vanillin	Use of certain species from the genus *Serratia*, *Klebsiella*, or *Enterobacter* to convert eugenol or isoeugenol to vanillin	G
	Kraft General Foods	7 Jul 1992	US 5128253 A	Bioconversion process for the production of vanillin	Use of ferulic acid-degrading microorganisms such as *Aspergillus niger*, *Rhodotorula glutinis*, or *Corynebacterium glutamicum* to convert ferulic acid to vanillin	G
	Firmenich	7 Nov 1995	US 5464761 A	Process for the enzymatic preparation of aliphatic alcohols and aldehydes from linoleic acid or a natural precursor	Use of lipoxygenase-containing soya flour and lyase-containing guava homogenate to convert linoleic acid to hexanal and related aldehydes	G
	BASF	19 May 1998	US 5753471 A	Biotechnological preparation of alcohols, aldehydes, and carboxylic acids	Use of isolated microorganisms capable of converting alkyl, alkenyl, aryl, and related compounds to their oxidized forms, including aldehydes	G
In vitro conversion of acid substrates using purified carboxylic acid reductases	University of Iowa	18 Aug 1998	US 5795759 A	Carboxylic acid reductase, and methods of using same	A purified carboxylic acid reductase (Car) enzyme from *Nocardia iowensis*, and use of it to convert vanillic acid to vanillin	G
	Michigan State University	16 April 2002	US 6372461 B1	Synthesis of vanillin from a carbon source	Use of an engineered microbe expressing recombinant DHSD and COMT as part of a metabolic pathway from glucose to vanillic acid, followed by reduction of vanillic acid to vanillin using a purified Car	G

	University of Iowa	16 September 2008	US 7425433 B2	Carboxylic acid reductase polypeptide, nucleotide sequence encoding same and methods of use	Use of Car to convert aromatic, aliphatic, and acyclic carboxylic acids to corresponding aldehydes	G
	Archer-Daniels-Midland	17 February 2009	US 7491854 B2	Enzymatic method of making aldehydes from fatty acids	Use of Car to convert fatty acids ranging from C6-C32 to corresponding aldehydes	G
De novo synthesis using engineered microbes harboring recombinant aldehyde biosynthetic genes (e.g., *car*, *aar*, and *kivD*)	DuPont	29 August 2006	US 7098000 B2	Method for production of C30-aldehyde carotenoids	Use of an engineered microorganism to convert fermentable carbon sources to diaponeurosporene monoaldehyde, diapocarotene monoaldehyde, or diapocarotene dialdehyde	G
	LS9	17 January 2012	US 8097439 B2	Methods and compositions for producing fatty aldehydes	Use of engineered microbes containing recombinant Car homologues to convert carbohydrates to aliphatic aldehydes	G
	LS9	18 September 2012	US 8268599 B2	Method for producing a fatty alcohol or fatty aldehyde	Use of acyl-ACP reductases to convert acyl-ACPs to aliphatic aldehydes	G
	International Flavors & Fragrances, and Evolva	14 February 2013	WO 2013022881 A1	Compositions and methods for the biosynthesis of vanillin or vanillin-beta-D-glucoside	Use of a microbe expressing recombinant AROM and/or COMT to convert glucose to vanillin or vanillin-beta-D-glucoside	A
	University of California	27 December 2013	WO 2013192237 A1	*Escherichia coli* engineered for isobutyraldehyde production	Use of an *E. coli* strain with reduced isobutyraldehyde reductase activity to accumulate isobutyraldehyde	A
	Easel Biotechnologies	9 January 2014	US 20140011231 A1	Microbial synthesis of aldehydes and corresponding alcohols	Use of an engineered microbe to convert glucose to short fatty aldehydes, followed by removal of aldehydes from the fermentation medium and conversion to alcohols *ex vivo*	A
	Genomatica	24 April 2014	WO 2014062564 A1	Microorganisms and methods for production of specific length fatty alcohols and related compounds	Use of a microbe expressing malonyl-CoA independent (or dependent) fatty acyl-CoA elongation pathways to produce fatty acids, aldehydes, and alcohols	A
	Evolva	4 September 2014	US 20140248668 A1	Methods and materials for recombinant production of saffron compounds	Use of a microorganism expressing recombinant pathways to convert glucose to picrocrocin, safranal, crocin, crocetin, or crocetin esters	A

[a] AROM, arom multifunctional enzyme; COMT, catechol-*O*-methyltransferase; DHSD, 3-dehydroshikimate dehydratase.

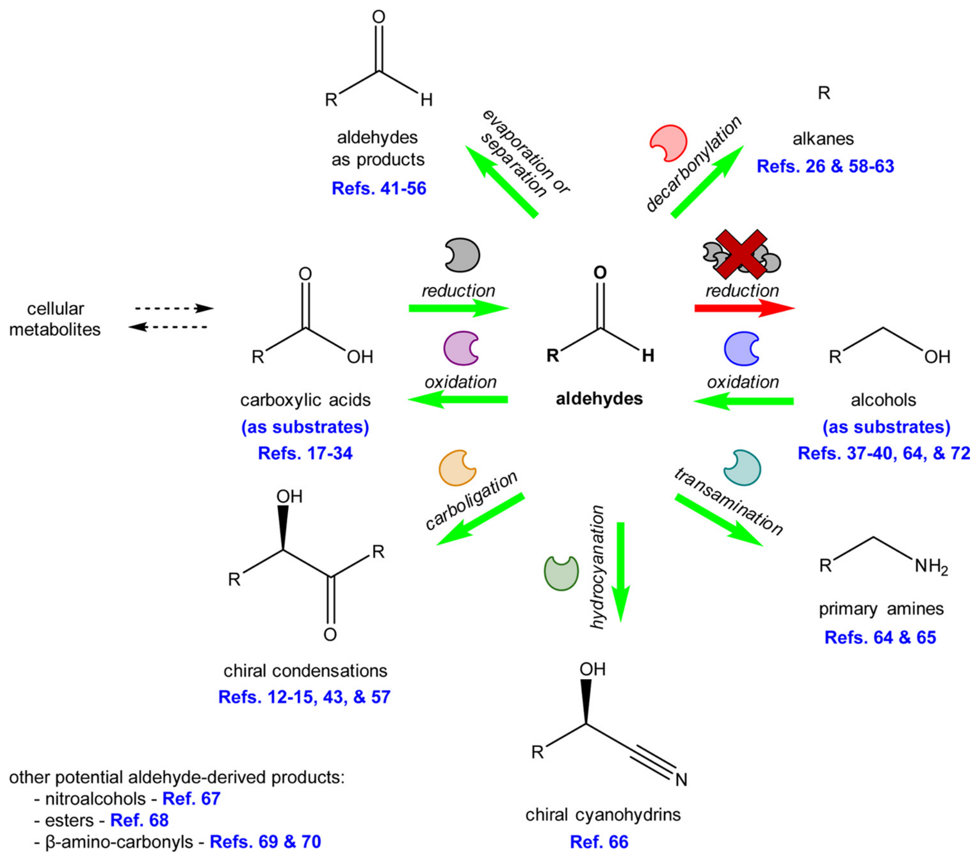

FIG 2 Potential biocatalytic and metabolic engineering opportunities enabled by, or enhanced by, microbial aldehyde accumulation.

dehydes remain toxic even when minimal endogenous reduction occurs (43).

A deeper understanding of precisely how aldehydes cause harm to cells may enable engineering strategies to surmount particular modes of toxicity. Certain aldehydes may be involved in mechanisms of toxicity that are far more detrimental than the mechanisms seen with others. For example, acetaldehyde has been shown to induce single-strand and double-strand breaks in DNA (80). Several aliphatic aldehydes are products of lipid peroxidation and have been implicated in the formation of adducts on a variety of biological macromolecules and as second messengers of reactive oxygen species (ROS) (81–83). However, the precise relationship between aldehydes and ROS is unclear. For example, it was recently shown that resistance of *E. coli* to exogenous methylglyoxal is conferred by decreased expression of *sodC* (84). This is a surprising result given that *sodC* encodes a superoxide dismutase, which breaks down ROS (85). There are numerous other potential mechanisms of aldehyde toxicity. Given the importance of lignocellulose utilization, potential mechanisms of toxicity for furfural in particular have been extensively reviewed and include mechanisms not described here (86, 87).

Until precise mechanisms of aldehyde toxicity are elucidated, there are some general engineering strategies that can be employed. Some bacteria have naturally evolved solutions to aldehyde toxicity beyond rapid reduction of aldehydes, such as protein microcompartments that feature aldehyde intermediates (88, 89). If control of selective metabolite transport through the protein shells were achieved, then the engineering of these compartments for biosynthesis of new aldehyde-derived products might aid in limiting the pool size of free aldehyde intermediates (90). Independently of the mode of toxicity, *in situ* separation using stripping (91), two-phase systems (92), or selective resins (93) may result in increased production of aldehydes as end products. Many aldehydes of interest are hydrophobic and volatile, which are properties that aid separation from water-based fermentation processes. In the event that precise mechanisms of aldehyde toxicity become known and prove to be insurmountable problems, then efforts should shift toward microbial engineering of aldehyde intermediates for synthesis of aldehyde-derived products.

CONCLUSION

In the past decade, research on microbial engineering for aldehyde synthesis has progressed from understanding how to synthesize aldehydes to understanding how to accumulate synthesized aldehydes. Given that advances in both of these areas apply to a broad range of societally relevant aldehydes, the work summarized here may serve as a foundation for future academic and commercial endeavors. The issue of aldehyde toxicity remains a major hurdle blocking improvement of commercial microbial processes for aldehyde production. Potential engineering solutions to this challenge are complicated by significant differences in the levels of toxicity among aldehydes and by the potential for each aldehyde to be deleterious due to multiple mechanisms acting at once. Regardless, given that aldehydes can now escape the fate of rapid reduction to their corresponding alcohols in living microbes, these molecules can serve as a gateway for synthesis of several previously challenging classes of biochemicals. For that reason at least, aldehydes should be of significant interest to practitioners of metabolic engineering and biocatalysis in the years ahead, even if the challenge of aldehyde toxicity has not been solved.

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