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MiniReview

Microbial aldo-keto reductases

Elizabeth M. Ellis *

Departments of Bioscience and Pharmaceutical Sciences, University of Strathclyde, 204 George Street, Glasgow G1 1XW, UK

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Abstract

The aldo-keto reductases (AKR) are a superfamily of enzymes with diverse functions in the reduction of aldehydes and ketones. AKR enzymes are found in a wide range of microorganisms, and many open reading frames encoding related putative enzymes have been identified through genome sequencing projects. Established microbial members of the superfamily include the xylose reductases, 2,5-diketo-D-gluconic acid reductases and β-keto ester reductases. The AKR enzymes share a common $(\alpha/\beta)_8$ structure, and conserved catalytic mechanism, although there is considerable variation in the substrate-binding pocket. The physiological function of many of these enzymes is unknown, but a variety of methods including gene disruptions, heterologous expression systems and expression profiling are being employed to deduce the roles of these enzymes in cell metabolism. Several microbial AKR are already being exploited in biotransformation reactions and there is potential for other novel members of this important superfamily to be identified, studied and utilized in this way.

Keywords: Aldo-keto reductase; Aldehyde; Ketone

1. Reduction of aldehydes and ketones in the microbial cell

Aldehydes and ketones are important intermediates in many metabolic pathways, including sugar metabolism, steroid biosynthesis, amino acid metabolism, and biosynthesis of secondary metabolites. In addition, microbial cells encounter aldehydic products of lipid peroxidation after exposure to oxidants and heavy metals [1], as well as directly from exogenous sources, including plant phytoallexins, toxins and synthetically derived chemicals in the environment. Because of the reactivity of the carbonyl group, many aldehydes and ketones are mutagenic representing a potential danger to microbial cells [2]. The ability to metabolize these compounds gives a selective advantage to any microorganisms that are exposed to them.

Aldehydes and ketones can be metabolized by several enzyme systems including aldehyde dehydrogenases that can oxidize aldehydes to acids, and the well-characterized alcohol dehydrogenases, which include the short-chain dehydrogenase/reductase and medium-chain dehydrogenase/reductase families (reviewed in [3,4]). The number and structural diversity of these latter enzyme families might be considered sufficient to account for the capacity of the cell to reduce aldehydes and ketones. However, members of another superfamily of enzymes, the aldo-keto reductases (AKR), are present in many microbial cells [5], suggesting an evolutionary convergence of function between the superfamilies. Knowledge of the AKR family has grown rapidly over the last decade, and it now contains over 60 members from across all species [6]. It is the intention of this minireview to present the current state of knowledge of the microbial members of the AKR superfamily, focussing in particular on the structural features and biochemical activities of the enzymes, how these might contribute to their physiological role within the cell, and the potential uses of the enzymes in biotransformation reactions.

2. The AKR superfamily

The aldo-keto reductases are a superfamily of related enzymes that share structural and functional features. Following rapid expansion of the superfamily, a systematic nomenclature was established based on amino acid similarity (reviewed in [7]), which is periodically updated [6].

* Tel.: +44 (141) 548 2122; Fax: +44 (141) 553 4124.
E-mail address: elizabeth.ellis@strath.ac.uk (E.M. Ellis).

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Enzymes have been divided into 14 families named AKR1–AKR14, and within these, subfamilies are denoted by a letter, with each enzyme having its own unique identifier. To be included, an enzyme has been shown to have activity or has been expressed. The majority of mammalian enzymes belong to the AKR1 family, but there is considerable diversity between the microbial enzymes, resulting in them spanning 10 families: AKR2 (comprising xylose reductases), AKR3, AKR5, and AKR8–AKR14. Characterized microbial AKR are listed in Table 1 and in addition to these 'known' enzymes, a further 200 putative AKR have been identified through genome sequencing projects [6]. Determining the abilities and functions of these enzymes in carbonyl metabolism is an important goal, but this task has proved difficult because of the wide substrate specificity of many of the AKR, and the typical presence of more than one AKR in each cell. However, it is possible that the flexibility of this family of enzymes to accept a wide range of substrates is the key to their persistence across phylogeny.

3. Structure of microbial AKR

Despite some convergence of function, the three-dimensional structure of AKR enzymes differs considerably from the short-chain dehydrogenase/reductase family [8]. In most cases, AKR are monomeric, with the exception of the dimeric mammalian AKR7 enzymes [9], and some dimeric yeast xylose reductases [10]. The structures for two microbial enzymes have been described. The first was 2,5-diketo-D-gluconic acid reductase (2,5-DKGR; AKR5D) from *Corynebacterium* sp. which catalyzes the NADPH-dependent stereospecific reduction of 2,5-diketo-D-gluco-

Table 1
Microbial members of the aldo-keto reductase superfamily

Division	Organism	Gene name	Enzyme or ORF name	AKR Name	Protein acc. no.	Sequence
Archaebacteria	*Haloferax volcanii*		oxidoreductase	AKR9C	AAB71807	–
Bacteria	*Bacillus subtilis*	*IOLS*	IolS	AKR11A	P46336	[52]
	Bacillus subtilis	*YHDN*	GSP69	AKR11B	P80874	[53]
	Corynebacterium sp.	*DKGA*	2,5-diketo-D-gluconic acid reductase	AKR5C	P06632	[54]
	Corynebacterium sp.	*DKGB*	2,5-diketo-D-gluconic acid reductase	AKR5D	P15339	–
	Escherichia coli	*yghZ*	aldo-keto reductase	AKR14A1	Q8X529	[30]
	Pseudomonas putida	*morA*	morphine 6-dehydrogenase	AKR5B	Q02198	[55]
	Saccharopolyspora erythraea	*eryBII*	mycarose/desosamine reductase	AKR12B	AAB84068	[42]
	Streptomyces avermitilis	*aveBVIII*	2,3-reductase	AKR12C	BAA84599	[56]
	Streptomyces bluensis	*blmT*	BlmT	AKR10A2	AAD28516	–
	Streptomyces fradiae	*tylCII*	2,3-enoyl reductase	AKR12A	AAD41821	[44]
	Streptomyces glaucescens	*strT*	StrT	AKR10A1	CAA07384	[57]
Yeasts	*Candida tenuis*	*xylR*	xylose reductase	AKR2B5	AF74484	[58]
	Candida tropicalis	*xyrA*	xylose reductase	AKR2B4	AB002106	[18]
	Candida tropicalis	*xyrB*	xylose reductase	AKR2B7	AB002105	[18]
	Kluveromyces lactis	*XYL1*	xylose reductase	AKR2B2	P49378	[19]
	Pachysolen tannophilus	*XYL1*	xylose reductase	AKR2B3	P78736	[59]
	Pichia guilliermondii	*Xyl1*	xylose reductase	AKR2B8	AF020040	[60]
	Pichia stipitis	*XYL1*	xylose reductase	AKR2B1	P31867	[17]
	Saccharomyces cerevisiae	*GRE3*	Gre3p; YHR104w	AKR2B6	NP_011971	[32]
	Saccharomyces cerevisiae	*GCY1*	Gcy1p; YOR120w	AKR3A1	P14065	[61]
	Saccharomyces cerevisiae	*YPR1*	Ypr1p; YDR368w	AKR3A2	NP_010656	[32]
	Saccharomyces cerevisiae	*ARA1*	arabinose dehydrogenase, YBR149w	AKR3C	Z36018	[32]
	Saccharomyces cerevisiae	*YDL124w*	YDL124w	AKR5E	NP_010159	[32]
	Saccharomyces cerevisiae	*YJR096w*	YJR096w	AKR5F	NP_012630	[32]
	Saccharomyces cerevisiae	*AAD14*	Aad14p; YNL331c	AKR9B1	NP_014068	[32]
	Saccharomyces cerevisiae	*AAD3*	Aad3p	AKR9B2	NP_010032	[32]
	Saccharomyces cerevisiae	*AAD4*	Aad4p	AKR9B3	NP_010038	[32]
	Saccharomyces cerevisiae	*AAD10*	Aad10p	AKR9B4	NP_012689	[32]
	Schizosacharomyces pombe	*PLR*	pyridoxal reductase	AKR8A1	T39218	[62]
	Schizosacharomyces pombe	*PLR*	pyridoxal reductase	AKR8A2	T40923	–
	Sporidiobolus salmonicolor	*ALR*	aldehyde reductase	AKR3B	U26463	[63]
Fungi	*Aspergillus parasiticus*	*norA*	norsolorinic acid reductase	AKR9A2	AAC49166	[46]
	Aspergillus nidulans	*STCV*	sterigmatocystin dehydrogenase	AKR9A1	Q00727	[45]
	Aspergillus niger	*XyrA*	xylose reductase	AKR2D	AAF61912	[64]
	Mucor mucedo	–	4-dihydromethyltrisporate dehydrogenase	AKR2C	Z73640	[65]
	Phanerochaete chrysosporium	*AAD*	aryl alcohol dehydrogenase	AKR9A3	Q01752	[27]
	Zymomonas mobilis	*dkg*	2,5-diketo-D-gluconic acid reductase	AKR5E	AAD42404	–

nate to 2-keto-L-gulonate, a precursor in the industrial production of vitamin C (L-ascorbate) [11] (Fig. 1). The second is a yeast enzyme, Gcy1p (AKR3A1) which can catalyze the reduction of several aldehyde substrates include D,L-glyceraldehyde, though its physiological function is unknown [12]. Both these enzymes are structurally similar to the mammalian AKR enzymes in that they share the characteristic $(\alpha/\beta)_8$ TIM barrel structure, and conserved active site residues (Fig. 1).

Structural information has revealed important details about the mechanism of catalysis which involves a catalytic tetrad of amino acids (Tyr, His, Asp and Lys), where the tyrosine is the general acid, and where there is thought to be a proton relay from the histidine or lysine. It appears that this mechanism is conserved as most active AKR identified to date possess these four amino acids (Fig. 1). The way in which AKR bind the cofactor NADPH in an extended conformation without a Rossman fold also appears to be conserved. In 2,5-DKGR, binding of NADPH causes a conformational change in the structure, and it has been suggested that this is essential for subsequent substrate binding [13].

Site-directed mutagenesis of mammalian AKR has revealed that the C-terminal region is important in determining the substrate specificity of the enzyme [14]. Studies using molecular modelling of microbial enzymes are underway with the aim of pinpointing those amino acids in the active site pocket that are responsible for substrate specificity and cofactor binding so that enzymes with enhanced characteristics can be designed [15].

4. Enzyme activities of microbial AKR

Of the AKR listed in Table 1, several have been studied with regards to their catalytic activities (Table 2). Other microbial enzymes with NADPH-dependent aldehyde or ketone reductase activity have been purified and assayed, but without any sequence data, it is not known whether they belong to the AKR superfamily or to one of the alcohol dehydrogenase families. Most of the AKR enzymes that have been characterized show a distinct preference for NADPH as cofactor; however, some of the yeast xylose reductases can use NADH and NADPH with equal efficiency. The range of substrates that individual AKR can reduce is usually quite broad, but there are clear differences in affinities (Table 2).

The xylose reductases from yeast form the AKR2B subfamily, and are reviewed comprehensively by Lee [16]. These enzymes catalyze the reduction of xylose to xylitol, as part of the pathway to xylulose, which can then feed into the pentose phosphate, Embden–Meyerhof or phosphoketolase pathways. They are found as expected in xylose-utilizing yeasts including *Pichia stipitis* [17], *Candida tropicalis* [18], and *Kluveromyces lactis* [19]. Intriguingly, *Saccharomyces cerevisiae* possesses an enzyme, Gre3p (AKR2B6), that is over 60% related to the xylose reductases, even though it is unable to utilize xylose and would therefore be expected to have no need for such an enzyme. In fact, the Gre3p enzyme has good activity towards D-xylose as well as other substrates [20,21], so its presence in the cell remains a mystery. Studies that have examined the

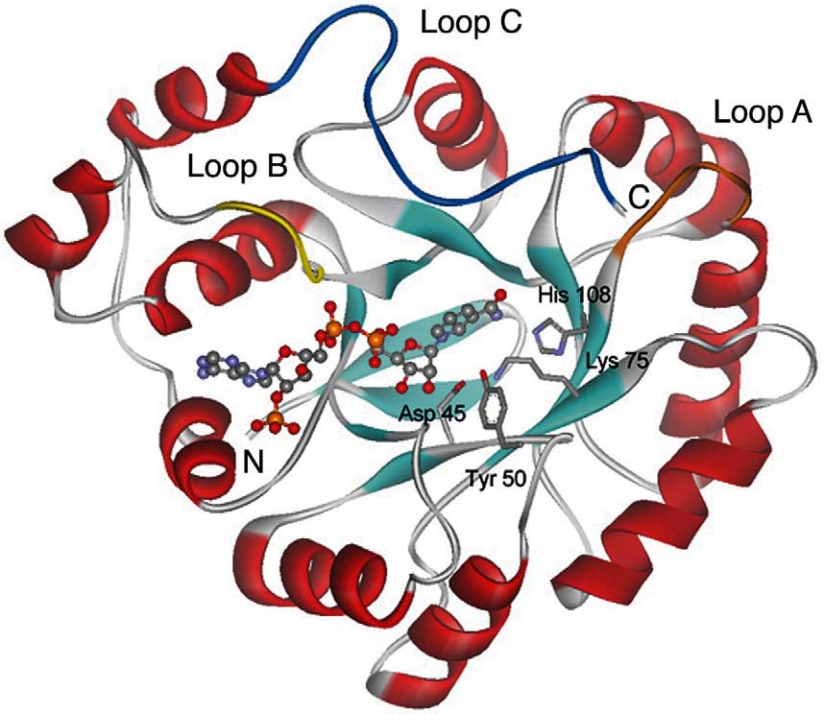

Fig. 1. Crystal structure of 2,5-diketo-D-gluconic acid reductase from *Corynebacterium* sp. [11]. View showing the $(\alpha/\beta)_8$ barrel structure and the position of the three loop regions. Bound cofactor NADPH is included and the four amino acids that form the catalytic tetrad are shown.

Table 2
Some substrate affinities of microbial AKR

Enzyme	Organism	AKR	K_m (mM)								
			4-NBA	D-xylose	D,L-GA	9,10-PQ	MeGly	NADPH (μM)	NADH (μM)	Other substrates (K_m)	Reference
Xylose reductase	*P. stipitis*	AKR2B1	–	42	18	–	–	9	21	L-arabinose (40 mM)	[66,67]
Xylose reductase	*P. tannophilus*	AKR2B3	0.37	25	18	–	–	16	NMA	D-glucose (>1000 mM)	[68]
Xylose reductase XRII	*C. tropicalis*	AKR2B4	–	37	–	–	–	18	NMA		[10,18]
Xylose reductase	*C. tenuis*	AKR2B5	0.38	76	2	–	6.3	16	195	benzaldehyde derivatives	[22,69]
Aldose reductase Gre3p	*S. cerevisiae*	AKR2B6	0.12	17	1.44	0.3	1.6	0.013	–		[20,21,25,39]
Xylose reductase XRI	*C. tropicalis*	AKR2B7	–	30	–	–	–	14	NMA		[10,18]
Gcy1p	*S. cerevisiae*	AKR3A1	0.13	NMA	11	–	–	28.5	NMA		[21,70]
Ypr1p	*S. cerevisiae*	AKR3A2	1.1	418	1.1	0.3	0.9	8.8	–	2-MBA (1.1 mM); ethyl 4-chloroacetoacetate (0.13 mM)	[71]; [25,26]
Aldehyde reductase	*S. salmonicolor*	AKR3B	0.8	403	–	–	3	–	–	ethyl 4-chloro-3-oxobutanoate	[72.63]
D-arabinose dehydrogenase	*S. cerevisiae*	AKR3C	–	–	–	–	–	–	–	L-xylose (24 mM); D-arabinose (161 mM)	[33]
Morphine dehydrogenase	*Pseudomonas* sp.	AKR5B	–	–	–	–	–	–	–	morphine (0.46 mM), codeine (0.04 mM)	[73]
2,5-DKGR-A	*Corynebacterium* sp.	AKR5C	–	–	–	–	–	10	–	2,5-DKG (26 mM), 5-keto fructose (155 mM), dihydroxyacetone (160 mM)	[11,54]
Pyridoxal reductase	*S. pombe*	AKR8A1	0.012	–	–	NMA	–	16	NMA	pyridoxal (0.9 mM)	[62]
Aad14p	*S. cerevisiae*	AKR9B1	0.95	NMA	–	0.02	2.7	–	–	hexenal (1 mM)	[25]
Aldo-keto reductase (YghZ)	*E. coli*	AKR14A1	1.06	–	–	–	3.24	–	–	isatin (2.05 mM)	[30]

4-NBA, 4-nitrobenzaldehyde; 9,10-PQ, 9,10-phenanthrenequinone; DL-Gla, D,L-glyceraldehyde; Me-Gly, methylglyoxal; 2-MBA, 2-methylbutryaldehyde; 2,5-DKG, 2,5-diketo-D-gluconic acid.
NMA, no measurable activity.

substrate specificity profiles of the xylose reductases from other yeast have shown that they too are capable of reducing other substrates. For example, a comprehensive study of the substrate specificity requirements of the *Candida tenuis* enzyme (AKR2B5) for benzaldehyde derivatives has been carried out [22]. It looks as if this enzyme, and perhaps other xylose reductases, has the capability to perform other additional functions within the cell.

Of the other yeast enzymes, Gcy1p and Ypr1 from *S. cerevisiae* were both thought to be glycerol dehydrogenases, based on their similarity to an *Aspergillus nidulans* glycerol dehydrogenase [23,24], but the in vitro derived enzyme data do not support this hypothesis. Ypr1p is a very poor glycerol dehydrogenase, but it does have high reductase activity towards 2-methylbutyraldehyde, which may represent an endogenous substrate in vivo [21,25,26]. Gcy1p has been shown to be able to reduce D,L-glyceraldehyde and 4-nitrobenzaldehyde (4-NBA) [12], but awaits further characterization in terms of its substrate specificity.

The aryl alcohol dehydrogenases (Aad; AKR9B subfamily) in *S. cerevisiae* form part of a closely related gene set that may have arisen through duplication at the telomeres. All are highly related to the aryl alcohol dehydrogenase of *Phanerochaete chrysosporium* which has been shown to reduce veratraldehyde (3,4-dimethoxybenzaldehyde) [27]. Out of the seven Aad open reading frames (ORFs) in *S. cerevisiae*, three have been expressed and studied in detail, of which only one (Aad14p) has been shown to possess any activity towards aldehyde and ketone substrates [25]. The existence of such non-functional genes or pseudogenes demonstrates the limitations of simply identifying homologous ORFs through genome sequencing projects, as this does not provide information about the function of the enzyme.

However, the use of genome sequencing data coupled with overexpression and enzyme characterization has led to the discovery of several novel bacterial AKR. YqhE from *Escherichia coli* was originally identified because of its similarity to 2,5-DKGR [28], and cloning and expression of this enzyme proved it to be active as an AKR. Recently, this enzyme has been shown to possess β-keto ester reductase activity [29]. Another *E. coli* enzyme (YghZ; AKR14A1) that showed similarity to mammalian AKR7 enzymes as well as to AKR6 potassium channel β-subunits has activity towards a range of substrates including methylglyoxal, and is able to contribute towards methylglyoxal detoxication in vivo [30].

5. Physiological roles of microbial AKR

Because of the overlapping substrate specificities of the AKR enzymes, it has often been difficult to assign functions to individual enzymes. In addition, there may be more than one enzyme within a single cell that is capable of reducing a particular aldehyde or ketone. For example, from microbial genome data, it can be predicted that a prokaryote such as *E. coli* possesses six AKR [31], and a simple eukaryote such as yeast has over 14 AKR [32]. This is in addition to the organism's complement of other dehydrogenases/reductases which can also perform similar functions in aldehyde metabolism [3,4]. A fundamental question arises: why does the cell have many enzymes capable of carrying out apparently similar roles? The answer is presumably that although there is considerable overlap in activities, each enzyme has evolved for a particular purpose, and its ability to handle a range of substrates belies the fact that it has within that group a preferred substrate.

5.1. Evidence of function from null mutants

To address the activity/function problem, several studies have used methods that disrupt or knock-out the gene in question. Deletion of the *ARA1* gene encoding D-arabinose dehydrogenase in *S. cerevisiae* gave convincing evidence of a role for this enzyme, as the resulting strain had no D-arabinose dehydrogenase activity [33]. Several other *S. cerevisiae* AKR genes have also been knocked out, including *YPR1* (AKR3A2), which has been shown to contribute 50% of in vivo 2-methylbutyraldehyde reductase activity [26]. Deletion of the *GRE3* gene encoding *S. cerevisiae* aldose reductase (AKR2B6) leads to a decrease in xylitol formation from xylose by 50% [34]. The latter two studies both suggest that there are other enzymes present in the cell that contribute to the remaining activity observed, which indicates at least partial functional redundancy.

Functional analysis was less successful in assessing the roles of the aryl alcohol dehydrogenase (*AAD*) genes in *S. cerevisiae*. A strain was constructed that contained multiple deletions of five of the *AAD* genes [35,36]. This strain showed little phenotypic differences from the wild-type, under the conditions tested [35,36]. Deletion of genes encoding three other AKR from *S. cerevisiae* (AKR5F, AKR5E, and AKR3A1 (Gcy1p)) have similarly shown little effect [21]. Disruption of the *norA* gene encoding norsolorinic acid dehydrogenase from *Aspergillus parasiticus* has also proved unsuccessful in demonstrating its role in aflatoxin biosynthesis, but this may be because of a gene duplication event [37]. A clearer understanding of the substrate specificities of enzymes, and of the conditions under which they are expressed, is needed so that appropriate conditions and substrates can be used to probe the knock-out phenotype in more detail.

5.2. Evidence of function from overexpression

In some cases, information on function has been gained from artificial overexpression of the enzyme, either native or heterologous, followed by examination of whether this

affects viability of the cell or alters its metabolism of specific compounds. This has been a favored approach with yeast, but more as a means of perverting metabolism rather than studying it. The use of the xylose reductases in this way is discussed later in this review. Several enzymes have also been overexpressed as a means of demonstrating their contribution to detoxication pathways. For example, overexpression of Gre3p in yeast cells leads to increased tolerance to methylglyoxal [34]. Similarly, the overexpression of AKR14A1 in *E. coli* can also give some protection against the same toxic dicarbonyl [30], suggesting that this enzyme may play a role in the metabolism/detoxication of this compound in vivo. It is worth noting that these latter experiments do not prove a role, merely put forward a physiological possibility.

5.3. Function inferred from regulation

Given the difficulties in interpreting the results from gene deletions, valuable information about an enzyme's function may be gleaned from understanding the physiological conditions under which it is expressed. This process will no doubt become easier with the availability of whole genome microarray data or whole cell proteome data, in which the expression of individual mRNAs or proteins can be followed globally. Expression of some of the *S. cerevisiae* AKR-encoding genes already discussed has been studied in some detail. For example, the *GRE3* gene (encoding AKR2B6) was identified by differential display polymerase chain reaction (PCR) as one of three *S. cerevisiae* genes that are highly inducible by osmotic stress [38]. *GRE3* mRNA is also inducible by hydrogen peroxide and ionic stress (LiCl) [38] and at the diauxic shift point, suggesting that it may have a role in metabolic adaptation/stress responses. It is possible therefore that the enzyme is required for the metabolism of specific sugars, or for the interconversion of compatible solutes for which metabolism of sugars is required. Alternatively, as it appears to be able to detoxify methylglyoxal, it may represent part of a detoxication pathway for this endogenous toxic aldehyde [39].

Levels of *GCY1* mRNA (encoding AKR3A1) and *YPR1* mRNA and its protein product AKR3A2 are also increased by osmotic stress [23,25,26] which led to the hypothesis that these enzymes act to dissimilate excess glycerol under salt conditions [40]. Transcription of the *GCY1* gene is also inducible by galactose, leading to a 20-fold increase in the levels of mRNA compared to the levels seen in glucose grown cells [41]. However, despite extensive data on this gene's regulation, its function is still unknown.

5.4. Evidence of function from co-regulation in biosynthetic clusters

The organization of co-expressed genes into biosynthetic clusters has provided some clues for the roles of enzymes involved in the biosynthesis of antibiotics, for example, in the synthesis of sugars required for the glycosylation of erythromycin and tylosin. The *eryBII* gene encoding an oxidoreductase from *Sacharopolyspora erythraea* (AKR12B) was identified as part of the erythromycin biosynthetic gene cluster in the pathway from NDP-4-keto-6-deoxy-D-glucose to NDP-L-mycarose [42] which was substantiated through analysis of metabolites in a strain specifically deleted for *eryBII* [43]. By analogy, the *tylCII* gene identified in the tylosin biosynthetic gene cluster, encoding AKR12A from *Streptomyces fradiae*, and *aveB-VIII*/*aveBI* from the avermectin cluster encoding AKR12C from *Streptomyces avermitilis*, are also thought to play roles as reductases in L-mycarose and L-oleandrose biosynthesis respectively [44], though a role for these latter two enzymes through the use of gene mutation/deletion has not been proven. Biosynthetic clusters have also been studied in fungal pathways: a sterigmatocystin dehydrogenase (AKR9A1) involved in the biosynthesis of this fungal secondary metabolite was identified as a transcript expressed under conditions that induce sterigmatocystin production [45]. Similarly, the role of the *norA* gene (encoding AKR9A2) from *A. parasiticus* as a norsolorinic acid dehydrogenase involved in aflatoxin biosynthesis was deduced from its co-regulation and with other adjacent genes in the cluster [46].

However, co-regulation studies need to be treated with some caution. The indication that the *Bacillus subtilis iolS* gene (encoding AKR11A) might be required for myo-inositol biosynthesis because it is cotranscribed with an inositol biosynthesis regulator in response to inositol, was not upheld. Subsequent construction of a strain in which the *iolS* gene had been deleted demonstrated that it is not essential for inositol biosynthesis [74].

6. Biotechnological uses of microbial AKR

Although the physiological function of many AKR remains unknown, several microbial enzymes have been purified and studied because of their ability to carry out unique biochemical transformations that have the potential for industrial or commercial use. One of the main aims of studying the yeast xylose reductases has been to allow their expression in a range of biotechnologically tractable species, with a view to generate strains which are capable of converting xylose to xylitol, a product which is used as a natural sweetener (reviewed in [47]). To increase xylitol yield, several studies have overexpressed xylose reductases from *P. stipitis* in *S. cerevisiae* [48], with some additional metabolic engineering required, particularly to overcome problems associated with redox balance/supply of reduced cofactors.

In addition, several microbial AKR have been used in biotransformations for the production of chiral synthons.

The use of microbial cells expressing high levels of specific AKR gives distinct advantages over whole cell biotransformations using endogenous enzymes at natural levels because using these engineered cells can reduce or eliminate racemic mixtures of products. For example, the stereoselective reduction of β-keto esters to chiral alcohols has been achieved through the expression of *Sporobolomyces salmonicolor* β-keto ester reductase (AKR3B) in *E. coli* [49], and there is the potential for using the recently identified *E. coli* β-keto ester reductase in a similar way [29]. Overexpression of Ypr1p (AKR3A2) coupled with gene deletions of AKR and other enzymes in *S. cerevisiae* has led to the production of stereospecific building blocks from β-keto esters [50].

At an enzyme level, attempts have been made to engineer the cofactor specificity of *Corynebacterium* sp. 2,5-diketo-D-gluconic acid reductase (AKR5C) for use in vitamin C biosynthesis, which on an industrial scale would be more economically viable if the enzyme could utilize NADH instead of NADPH [51].

7. Conclusions and future perspectives

The microbial aldo-keto reductases represent a diverse collection of enzymes that are involved in a range of cellular processes. For many known and putative enzymes investigations into physiological functions are underway, using a combination of approaches and technologies which attempt to link the activity of the enzyme to the physiological conditions under which it is expressed and is therefore likely to be required. Cloning and expression of ORFs encoding enzymes identified in microbial genomes through their similarities to known AKR is enabling their activities to be characterized. Expression profiling using whole genome microarrays has the potential to provide information on the regulation of previously uncharacterized AKR ORFs, giving some clues as to their physiological roles. Gene knock-outs and overexpression strategies can then be employed to investigate function in vivo. Understanding structure and function relationships between the existing family members will undoubtedly provide valuable information that can be used for rational enzyme design, and this has the potential for a variety of biotechnological applications involving aldehydes and ketones. Additionally, genome database mining for natural AKR that are likely to possess features that could be exploited could be achieved through modelling of putative ORFs coupled with a deeper understanding of substrate specificity determinants.

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