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MINI-REVIEW

Biochemical properties of retinoid-converting enzymes and biotechnological production of retinoids

Seung-Hye Hong[1] · **Kyoung-Rok Kim**[1] · **Deok-Kun Oh**[1]

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Abstract Retinoids are a class of compounds that are forms of vitamin A and include retinal, retinol, retinoic acid, and retinyl ester. Retinal is involved in visual cycle, retinol has anti-infective, anticancer, antioxidant, and anti-wrinkle functions, and retinoic acid is used to treat acne and cancer. Retinol, retinoic acid, and retinyl ester are used in cosmetic and pharmaceutical industries. In this article, the biochemical properties and active sites and reaction mechanisms of retinoid-converting enzymes in animals and bacteria, including retinol dehydrogenase, alcohol dehydrogenase, aldo-keto reductase, and aldehyde dehydrogenase, are reviewed. The production of retinoids, using retinoid-producing enzymes and metabolically engineered cells, is also described. Uncharacterized bacterial proteins are suggested as retinoid-converting enzymes, and the production of retinoids using metabolically engineered cells is proposed as a feasible method.

Keywords Retinoids · Retinoid-converting enzyme · Biotransformation · Enzyme characterization

Introduction

Retinoids are a class of compounds that are forms of vitamin A. They can exist in a variety of forms, including retinal, retinol, retinoic acid, and retinyl ester, which are the aldehyde, alcohol, carboxylic, and ester forms of vitamin A, respectively. Retinal is a precursor of retinol and is a necessary structural component of the light-sensitive pigment, rhodopsin in the retina cells (Bates 1995). Retinol has been utilized in the manufacturing of food products, cosmetics, pharmaceuticals, nutraceuticals, and animal feed additives because of their anti-infective, anticancer, antioxidant, and anti-wrinkle functions (De Luca 1991; Fisher and Voorhees 1996; Semba 1999). Retinoic acid is crucially important for development, cell differentiation, and cancer prevention. It can also protect the skin and has been used to treat acne and prevent certain skin cancers. Retinyl palmitate is a retinyl ester that has been used as vitamin A supplement, skin care product, and treatment for dry eye and vitamin A deficiency. Retinyl acetate, another retinyl ester, is used to fortify foods and is generally recognized as safe (GRAS) (Moon et al. 1977). The biological functions of retinoids and related enzymes in physiology and pathology have been studied by numerous researches. Most studies regarding retinoid-converting enzymes have dealt with only animal enzymes. Recently, some bacterial retinoid-converting enzymes have been identified. Thus, in the current review, we have focused on bacterial retinoid-converting enzymes.

The main objective of this review is to present the biochemical properties of retinoid-converting enzymes from animals and bacteria. We also review and compare the active sites and reaction mechanisms of retinoid-converting enzymes for retinoids. The production of retinol and retinoic acid from retinal by retinoid-converting enzymes, including retinol dehydrogenase (RDH), alcohol dehydrogenase (ADH), aldo-keto reductases (AKR), and aldehyde dehydrogenases (ALDH) and the conversion of retinol to retinyl ester by acyl-CoA retinol *O*-fatty acyltransferase (ARAT) are introduced. In addition, the production of retinal and retinol from glycerol by metabolically engineered cells, which was prepared from the introduction of foreign β-carotene 15,15′-

✉ Deok-Kun Oh
deokkun@konkuk.ac.kr

[1] Department of Bioscience and Biotechnology, Konkuk University, Seoul 143-701, Republic of Korea

oxygenase (BCO) gene into β-carotene-producing *E. coli*, is presented.

Biological activities of retinoids and their derivatives

The biological activities of retinoids and their derivatives are summarized in Table 1. 11-*cis*-Retinal is required for vision because it functions as a light-absorbing pigment in the retina (Wenzel et al. 2005). All-*trans*-retinol is used to treat the visible signs of premature aging, such as fine lines, age spots, and other types of pigmentation anomalies (Yoshimura et al. 2003). All-*trans*-retinoic acid is used for treating acne vulgaris, keratosis pilaris, photoaging, hair loss, and acute promyelocytic leukemia (Wang et al. 1999; Stefanaki et al. 2005; Rogers and Avram 2008). The proliferation of breast cancer cells is inhibited by 9-*cis*-retinoic acid because it binds to and activates various estrogen receptors (Moon 1994; Paik et al. 2005). A synthetic analog of vitamin A, 13-*cis*-retinoic acid, is used to treat cystic acne, skin disease, and brain tumor (see et al. 2004). All-*trans*-retinyl palmitate and all-*trans*-retinyl acetate are the ester forms of retinol attached to palmitate and acetate, respectively, and confer stability to vitamin A. Because of their stability, they are used as constituents of some topical skin care products (Moon et al. 1977; Yan et al. 2007).

Enzyme families involved in the conversion of retinoids

Retinoid can be transformed into an appropriate form (retinal, retinol, retinoic acid, or retinyl ester) in the organ by retinoid-converting enzymes during the metabolism (Fig. 1). BCO converts β-carotene to retinal, which is converted to retinoic acid by ALDH. All-*trans*-retinal, 9-*cis*-retinal, and 11-*cis*-retinal are reversibly converted to all-*trans*-retinol, 9-*cis*-retinol, and 11-*cis*-retinol, respectively, by AKR, ADH, and RDH. AKR from *Marivirga tractuosa* can irreversibly convert the retinals to their retinols (Hong et al. 2014a). All-*trans*-retinal is converted to 11-*cis*-retinal by light, isomerase, or esterase. ARAT converts retinol to retinyl ester.

Retinoid-converting enzymes from animals and bacteria are presented by the classification of the enzyme family RDH, ADH, AKR, ALDH, and ARAT (Table 2). RDH (EC 1.1.1.105) converts retinal to retinol with NADH or NADPH as a cofactor and also converts retinol to retinal with NAD^+ or $NADP^+$. ADH (EC 1.1.1.1) is a medium-chain alcohol dehydrogenase containing an active site with a catalytic zinc molecule (Persson et al. 1994) because ADH facilitates the conversion between alcohols and aldehydes and can catalyze the NAD(P)H-dependent reduction of retinal to retinol (Boleda et al. 1993; Yang et al. 1994) and the NAD^+−dependent oxidation of retinol to retinal (Ryan 1967). AKR (EC 1.1.1.2) is a NADPH-dependent oxidoreductase with wide substrate specificities for carbonyl compounds (Bohren et al. 1989). This enzyme family belongs to the short-chain dehydrogenase/reductase (SDR) superfamily. Within the AKR family, only AKR1B1, AKR1B10, and AKR1C4 and AKR from *M. tractuosa* can convert retinal to retinol (Ruiz et al. 2011, 2012; Hong et al. 2014a). ALDH (EC 1.2.1.3) irreversibly catalyzes the $NAD(P)^+$-dependent oxidation (dehydrogenation) of aldehyde to carboxylic acid (Yoshida et al. 1998). ALDHs from human, bovine, murine tissues, rats, sheep, and mice have catalyzed the oxidation of retinal to retinoic acid (Bhat et al. 1995; Saari et al. 1995; Moore et al. 1998; Lin et al. 2003; Vella et al. 2011). ARAT (EC 2.3.1.76) catalyzes the conversion of the two substrates acyl-CoA and retinol to its two products CoA and retinyl ester. ARATs from human and bovine convert all-*trans*-retinol and 11-*cis*-retinol to all-*trans*-retinyl ester and 11-*cis*-retinyl ester, respectively (Orland et al. 2005; Kaschula et al. 2006).

Biochemical properties of retinoid-converting enzymes

The optimal pHs for RDH, ADH, AKR, and ARAT are in a range of 7.4–7.6, with the exception of *Kangiella koreensis* ADH (pH 6.5) (Table 3). The optimum pH for mammalian ALDHs is 8.5, while that for ALDH from *Bacillus cereus* is

Table 1 Biological activities of retinoids and their derivatives

Retinoid	Substrate	Enzyme	Biological activity	Reference
11-*cis*-Retinal	All-*trans*-retinal	RDH	Chromophore of opsins in retina for vision	(Wenzel et al. 2005)
All-*trans*-retinol	All-*trans*-retinal	ADH, SDR, AKR	Treatment for the visible signs of premature aging	(Yoshimura et al. 2003)
All-*trans*-retinoic acid	All-*trans*-retinal	ALDH	Treatment for keratosis, photoaging, hair loss, and leukemia	(Wang et al. 1999)
9-*cis*-Retinoic acid	9-*cis*-Retinal	ALDH	Treatment for cancer and acne	(Paik et al. 2005)
13-*cis*-Retinoic acid	13-*cis*-Retinal	ALDH	Treatment for cystic acne, skin disorders, and brain tumor	(See et al. 2004)
All-*trans*-retinyl palmitate	All-*trans*-retinol	ARAT	Treatment for vitamin A deficiency, antioxidant activity	(Yan et al. 2007)
All-*trans*-retinyl acetate	All-*trans*-retinol	ARAT	Antineoplastic and chemopreventive activities	(Moon et al. 1977)

RDH retinol dehydrogenase, *ADH* alcohol dehydrogenase, *SDR* short-chain dehydrogenase, *AKR* aldo-keto reductase, *ALDH* aldehyde dehydrogenase, *ARAT* acyl-CoA retinol *O*-fatty acyltransferase

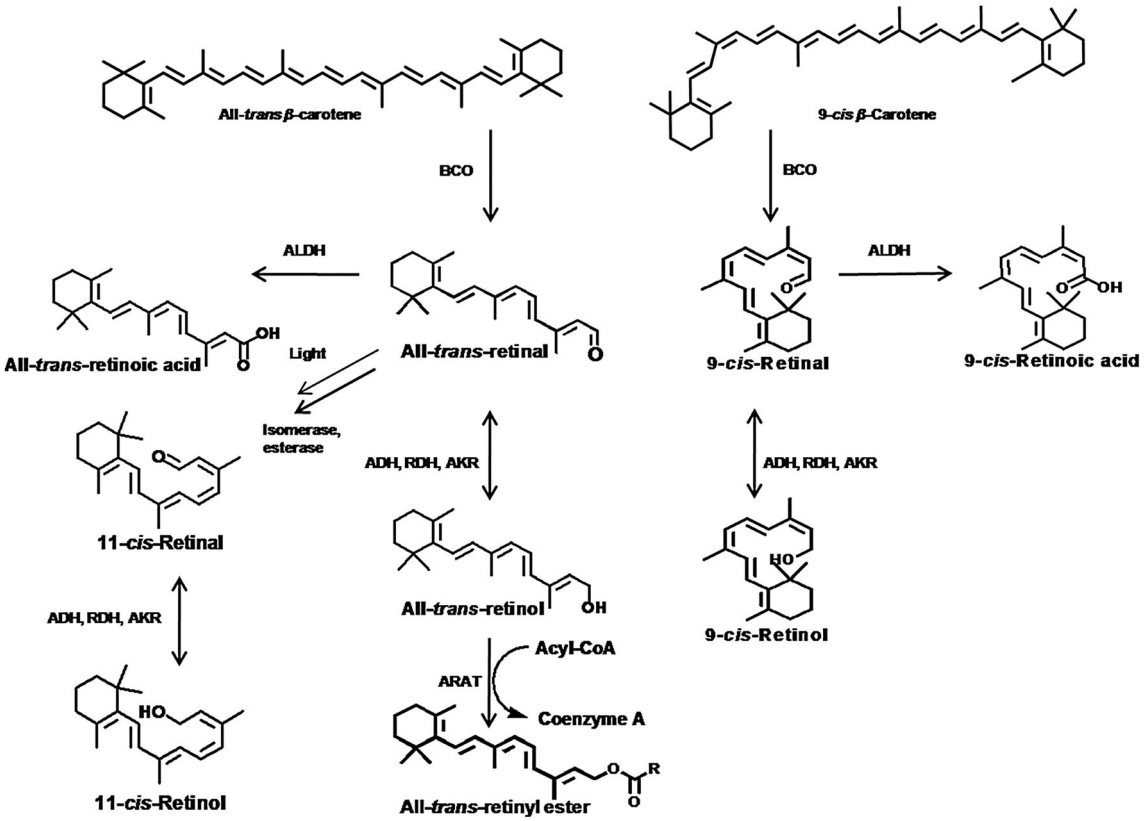

Fig. 1 Retinoid metabolism catalyzed by retinoid-producing enzymes. *BCO* β-Carotene oxygenase, *ADH* alcohol dehydrogenase, *RDH* retinol dehydrogenase, *AKR* aldo-keto reductase, *ALDH* aldehyde dehydrogenase, *ARAT* acyl-CoA retinol *O*-fatty acyltransferase

7.0. The optimal temperatures of retinoid-converting enzymes are in a range of 25–40 °C, with the exception of *K. koreensis* ADH (65 °C). RDH and AKR prefer NADPH/$NADP^+$ to NADH/NAD^+ as a cofactor, whereas ADH prefers NADH/NAD^+ to NADPH/$NADP^+$, except for frog ADH8. ALDH requires only NAD^+.

The kinetic parameters of retinoid-converting enzymes for retinoids as substrates are presented in Table 3. The catalytic efficiencies (k_{cat}/K_m) of all-*trans*-retinal, 9-*cis*-retinal, all-*trans*-retinol, and 9-*cis*-retinol show highest for AKR1B10, ADH1B2, ADH4 (Gallego et al. 2006), and AKR1C3 (Ruiz et al. 2011) from human, respectively. The turnover numbers (k_{cat}) of all-*trans*-retinal and 9-*cis*-retinal are highest for frog ADH8 (Peralba et al. 1999), whereas those of all-*trans*-retinol and 9-*cis*-retinol are highest for human ADH4 (Gallego et al. 2006) and AKR1C3 (Ruiz et al. 2011), respectively. The k_{cat}/K_m of frog ADH8 follows the order all-*trans*-retinal > 9-*cis*-retinal > 9-*cis*-retinol. The K_m and k_{cat} of *M. tractuosa* AKR for all-*trans*-retinal are higher than those of *K. koreensis* ADH, whereas k_{cat}/K_m of *M. tractuosa* AKR is lower. The k_{cat}/K_m and k_{cat} of rat ALDHA2, in the oxidation of all-*trans*-retinal to all-*trans*-retinoic acid, are highest among the ALDHs. They are 21- and 75-fold higher than those of *B. cereus* ALDH, respectively. The specific activity of *M. tractuosa* AKR for all-*trans*-retinal is highest, suggesting greater potential for bacterial enzymes in industrial application than their animal counterparts.

Active sites and mechanisms of retinoid-converting enzymes

RDH, ADH, and AKR commonly have a catalytic residue that contains a hydroxyl group as a functional group at the active site (Fig. 2). The catalytic residue is Tyr for RDH and AKR, or Thr for ADH. RDH and AKR have Lys as a helper residue, which assists in the activation of Tyr. Cys in ALDH is the first acting catalytic residue for nucleophilic attack, and Glu plays a role as a catalytic base, activating the catalytic Cys residue.

The proposed active-site residues (Asn14, Arg15, Gly16, Arg38, Arg42, Ile138, Ser140, Tyr194, Lys198, and Pro228) for the homology model of *M. xanthus* RDH are constructed based on the structure of human RDH (PDB code 1YDE) with an amino acid sequence identity of 40 % (Fig. 2a). Tyr194, the catalytic residue, forms a hydrogen bond with the hydroxyl group of all-*trans*-retinol as a substrate. Lys198 has dual roles; it is involved in the orientation of the nicotinamide nucleotide moiety in the cofactor and in the lowering of the pKa value of Tyr194 (Takahashi et al. 2009). *K. koreensis* ADH docked with the cofactor NADH (PDB code in process), and its active

Table 2 Animal and bacterial enzyme families involved in the conversion of retinoids

Division	Organism	Family	Enzyme name	Gene name	Protein acc. no	PDB	Reference
Animal	Human	RDH	Retinol dehydrogenase 12	Rdh12	EAW80951.1		(Haeseleer et al. 2002)
	Mouse		Retinol dehydrogenase 12	Rdh12	AAH16204.1		(Kanan et al. 2008)
	Bovine		Retinol dehydrogenase 12	Rdh12	AAM51556.1		(Haeseleer et al. 2002)
	Human		Short-chain dehydrogenase/reductase 3	Dhrs3	BAG35800.1		(Cerignoli et al. 2002)
	Mouse		Short-chain dehydrogenase/reductase 3	Dhrs3	BAC28098.1		(Haeseleer et al. 1998)
	Bovine		Short-chain dehydrogenase/reductase 3	Dhrs3	AAI04576.1		(Haeseleer et al. 1998)
	Rat		Retinol dehydrogenase 2	Rdh2	AAH79153.1		(Chai et al. 1997)
	Mouse		11-*cis*-Retinol dehydrogenase	Rdh5	AAC00492.1		(Gamble et al. 1999)
	Human		Retinol dehydrogenase 16	Rdh16	AAC72923.1.		(Lapshina et al. 2003)
	Mouse	ADH	Alcohol dehydrogenase 1	Adh1	AAH13477.1		(Zgombic-Knight et al. 1995)
	Human		Alcohol dehydrogenase 4	Adh4	BAG58459.1	3COS	(Martras et al. 2004a)
	Mouse		Alcohol dehydrogenase 4	Adh4	BAE21459.1	1E3E	(Martras et al. 2004b)
	Mouse		Alcohol dehydrogenase class-3	Adh5	BAE27558.1	1OTQ	(Zgombic-Knight et al. 1995)
	Human		Alcohol dehydrogenase class 4 mu/sigma chain	Adh7	BAG63168.1	1AGN	(Xie et al. 1997)
	Mouse		Alcohol dehydrogenase class 4 mu/sigma chain	Adh7	AAA76735.1		(Xie et al. 1997)
	Frog		NADP-dependent alcohol dehydrogenase	Adh8	CAA05553.1	1P0C	(Peralba et al. 1999)
	Human	AKR	Aldose reductase family 1 member B1	Akr1b1	CAA33460.1	2FZD	(Ruiz et al. 2012)
	Human		Aldo-keto reductase family 1 member B10	Akr1b10	AAP35440.1	1ZUA	(Gallego et al. 2007)
	Human		Aldo-keto reductase family 1 member C4	Akr1c4	BAA92885.1	2FVL	(Ruiz et al. 2011)
	Human	ALDH	Retinal dehydrogenase 1	Aldh1a1	AAR92229.1		(Yoshida et al. 1993)
	Rat		Retinal dehydrogenase 1	Aldh1a1	AAC53304.1		(Bhat et al. 1995)
	Mouse		Aldehyde dehydrogenase family 8 member A1	Aldh8a1	BAE25524.1		(Lin et al. 2003)
	Bovine		Retinal dehydrogenase 1	Aldh1a1	ABS44983.1		(Saari et al. 1995)
	Sheep		Retinal dehydrogenase 1	Aldh1a1	AAA85435.1	1BXS	(Moore et al. 1998)
	Bovine	ARAT	Lecithin retinol acyltransferase	LRAT	AAK08701.1		(Kaschula et al. 2006)
	Human	ARAT	Lecithin retinol acyltransferase	LRAT	EAX04904.1		(Orland et al. 2005)
	Chicken	ARAT	Lecithin retinol acyltransferase	Lrat	BAB23696.1	4Q95	(Nagatsuma et al. 2009)
Bacteria	*Myxococcus xanthus*	RDH	Oxidoreductase, short-chain dehydrogenase/reductase family	Mxan_4206	ABF86357.1		This study
	Alicyclobacilus acidocaldarius		Short-chain dehydrogenase/reductase	Aaci_0216	ACV57279.1		This study
	Kangilella koreensis	ADH	*S*-(Hydroxymethyl)glutathione dehydrogenase	Kkor_2048	ACV27458.1	Process	(Hong et al. 2015)
	Aeromonas hydrophilia		Alcohol dehydrogenase class 3	Aha_0214	ABK39411.1		This study
	Marivirga tractuosa	AKR	Aldehyde reductase	Ftrac_3685	ADR23652.1		(Hong et al. 2014a)

Table 2 (continued)

Division	Organism	Family	Enzyme name	Gene name	Protein acc. no	PDB	Reference
	Croceibacter atlanticus		Aldehyde reductase	Ca2559_11288	EAP86616.1		This study
	Bacillus cereus		Glyoxal reductase	C2mv60_BACCE	KFL76128.1		This study
	Bacillus cereus	ALDH	Aldehyde dehydrogenase	Bcere0002_25940	KFL74159.1	4PT0	(Ngo et al. 2013)

site comprises of Zn^{2+}, along with Cys45, Thr47, His67, Cys174, Gly175, Thr178, Ile203, Ile292, Gly293, and Val294 (Fig. 2b). Thr47 acts as a catalytic residue, while Zn^{2+} coordinates all-*trans*-retinal, Cys45, Cys174, His67, His67, Gly293, Ile292, and Val294, stabilizing NADH through the formation of forming hydrogen bonds. The proposed active-site residues (Asp43, Ala45, Tyr48, Lys77, His110, Ala158, Lys212, and Gln270) of the homology model of *M. tractuosa* AKR are constructed based on the structure of human AKR1B10 (PDB code 1ZUA) with an amino acid sequence identity of 46 % (Fig. 2c). A catalytic tetrad (Tyr48, Asp45, Lys77, and His110) is involved in the dehydrogenase of the aldehyde group of all-*trans*-retinal. The active site of *B. cereus* ALDH with NAD^+ (PDB code 4PT0) is composed of Phe169, Val172, Met173, Lys191, Ala193, Glu194, Gly244, Glu266, Cys300, and Glu457 (Fig. 2d), where Glu266 and Cys300 are the catalytic residues.

The reaction mechanisms of retinoid-converting enzymes are presented in Fig. 3. At the first step of the RDH reaction, the protonated phenolic group of the conserved Tyr residue forms a hydrogen bond with the hydroxyl group of the substrate (retinol) (Fig. 3a). The hydroxyl group of the conserved Ser residue also forms a hydrogen bond with the substrate, stabilizing its position. At the second step, a Tyr anion acts as a catalytic base to extract a proton from the hydroxyl group of the substrate. Concomitantly, $NADP^+$ accepts a proton liberated from the substrate to the nicotinamide ring, resulting in the production of retinal and NADPH. The ADH reaction commences with the binding of NAD^+, the coordination of the alcohol substrate with Zn^{2+} (Vallee and Hoch 1955), the deprotonation of the substrate, the hydride transfer to NAD^+ by Ser, and the formation of a complex between alkoxide ion and Zn^{2+} at the active site. This is followed by the release of retinal and the dissociation of NADH (Theorell 1961) (Fig. 3b). The AKR reaction is induced by a conserved tetrad of active-site residues (Tyr, Lys, Asp, and His), with Tyr acting as the proton donor (Hur and Wilson 2000). Thus, a hydrogen-bonding interaction between the phenolic hydroxyl group of Tyr and the ammonium side chain of Lys facilitates hydride transfer (Fig. 3c). The oxidation reaction of ALDH involves the sequential binding of NAD^+ and retinal, which is catalyzed by Cys to form a thiohemiacetal intermediate (MacGibbon et al. 1977) (Fig. 3d). Transfer of the hydride to the nicotinamide ring of NAD^+ results in the formation of a thiol ester intermediate. This intermediate is hydrolyzed by a water molecule and is then activated by Glu (Wang and Weiner 1995). As a result, retinoic acid and NADH are released (Graham et al. 2006).

Production of retinoids by retinoid-producing enzymes

The production of retinoids by RDH, ADH, AKR, ALDH, and BCO has been previously investigated under aqueous

Table 3 Kinetic parameters of retinoid-converting enzymes for retinoids as substrates

Substrate	Product	Enzyme	Source	Cofactor	Temp. (°C) /pH	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m (mM^{-1} min^{-1})	Specific activity (nmol min^{-1} mg^{-1})	Reference
All-*trans*-retinal	All-*trans*-retinol	RDH 10[a]	Human	NADPH	37/7.4	570			1.1	(Takahashi et al. 2009)
		RDH[a]	*M. xanthus*	NADPH	40/7.5	2700	1.3	0.5	25	This study
		PSDR1[a]	Human	NADPH	25/7.5	0.5			18	(Kedishvili et al. 2002)
		ADH8[b]	Frog	NADPH	25/7.5	8.0	270	33,750		(Peralba et al. 1999)
		ADH[a]	*K. koreensis*	NADH	60/6.5	1.8	1.7	944	22	(Hong et al. 2014b)
		AKR1B10[a]	Human	NADPH	25/7.5	0.6	27	45,000	300	(Gallego et al. 2006)
		AKR[a]	*M.tractuosa*	NADPH	30/7.5	48	22	427	319	(Hong et al. 2014a)
9-*cis*-Retinal	9-*cis*- Retinol	RDH 10[a]	Human	$NADP^+$	37/7.6	4.1			3.4	(Takahashi et al. 2009)
		PSDR1[a]	Human	NADPH	25/7.5	0.2			1.6	(Kedishvili et al. 2002)
		ADH8[b]	Frog	NADPH	25/7.5	9.0	70	7800		(Peralba et al. 1999)
All-*trans*-retinol	All-*trans*-retinal	AKR1C3[a]	Human	NADPH	37/7.4	0.4	13	32,500	193	(Ruiz et al. 2011)
		AKR[a]	*M.tractuosa*	NADPH	30/7.5	225	1.6	8.0	43	(Hong et al. 2014a)
		RDH10[a]	Human	NAD^+	37/7.4	4.1			0.06	(Takahashi et al. 2009)
		PSDR1[a]	Human	NADPH	25/7.5	1.3			1.0	(Kedishvili et al. 2002)
		ADH[a]	*K. koreensis*	NAD^+	60/6.5	0.3	1.9	5590	24	(Hong et al. 2014b)
		AKR[a]	Chicken	$NADP^+$	25/7.5	25	2.1	80	31	(Crosas et al. 2001)
9-*cis*- Retinol	9-*cis*- Retinal	ADH8[b]	Frog	$NADP^+$	25/7.5	19	5.7	300		(Peralba et al. 1999)
		AKR1C3[a]	Human	NADPH	37/7.4	0.4	13	32,500	193	(Ruiz et al. 2011)
All-*trans*-retinal	All-*trans*-retinoic acid	RALDH3[a]	Mouse	NAD^+	37/8.5	3.9			306	(Sima et al. 2009)
		ALDH1A1[c]	Human	NAD^+	25/8.5	8.1	4.2	519		(Bchini et al. 2013)
		ALDH1A2[c]	Rat	NAD^+	25/8.5	2.0	6.0	3000		(Bchini et al. 2013)
		ALDH[a]	*B. cereus*	NAD^+	37/7.0	7.0	0.3	40	0.1	This study
9-*cis*-Retinal	9-*cis*-Retinoic acid	RALDH4[a]	Mouse	NAD^+	37/8.5	3.0			81	(Sima et al. 2009)
13-*cis*- Retinal	13-*cis*-Retinoic acid	RALDH4[a]	Mouse	NAD^+	37/8.5	3.4			28	(Sima et al. 2009)
All-*trans*-retinol	All-*trans*-retinyl ester	ARAT[a]	Human		37/7.4	25.9			0.84	(Orland et al. 2005)
11-*cis*-Retinol	11-*cis*-Retinyl ester	ARAT[a]	Bovine		37/7.5	8.7				(Kaschula et al. 2006)

[a] The products formed at different reaction times during the reactions were analyzed by a HPLC system with standards

[b] The initial velocities of the substrates were determined by monitoring the formation (ε_{400} = 29.5 mM^{-1} cm^{-1}) or reduction (ε_{425} = 14.6 mM^{-1} cm^{-1}) of all-*trans*-retinal using a UV-visible spectrophotometer

[c] The reactions were monitored by NADH fluorescence using a stopped-flow apparatus

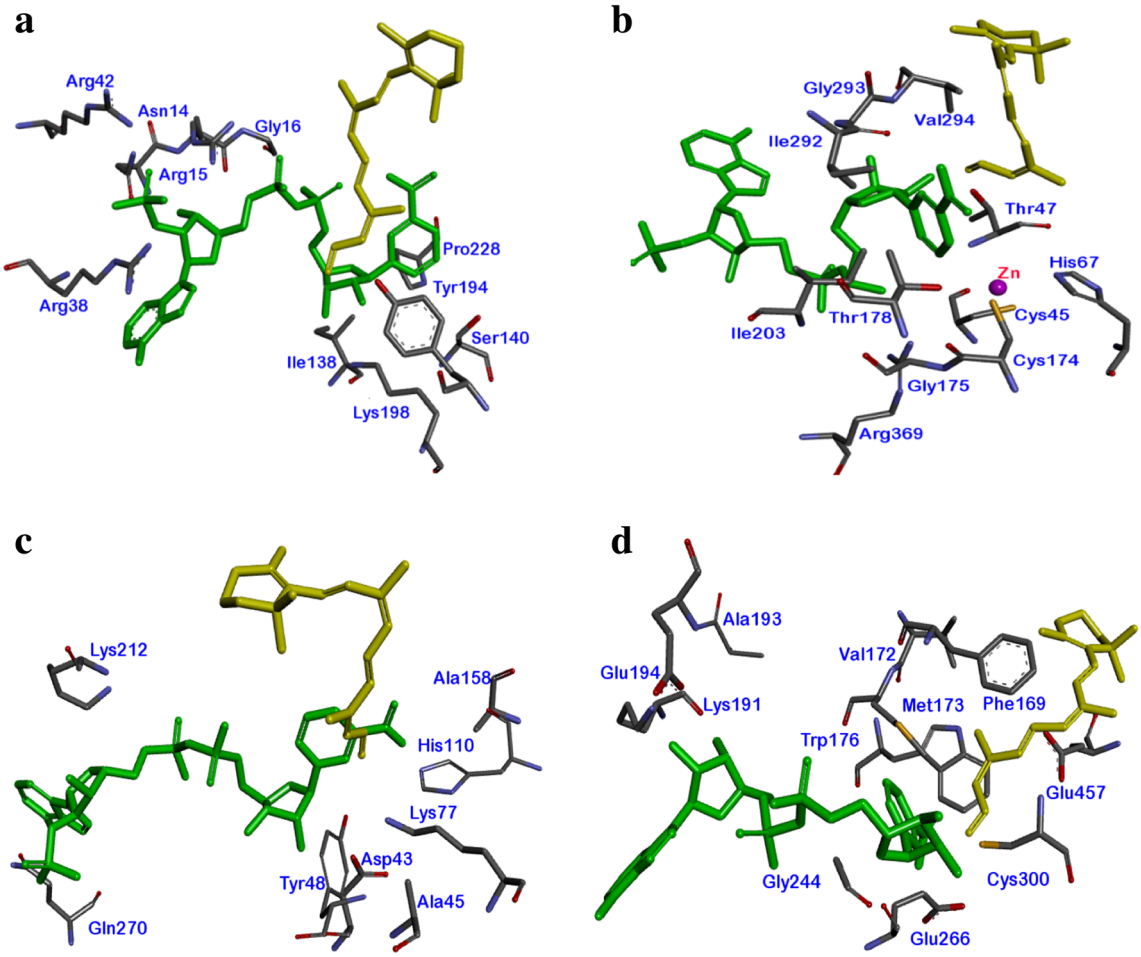

Fig. 2 Active-site structures of retinoid-converting enzymes. **a** RDH from *M. xanthus*. All-*trans*-retinal and nicotinamide of the enzyme-NADPH complex are shown in *yellow* and *green*, respectively. **b** ADH from *K. koreensis*. All-*trans*-retinal and NADH are shown in *yellow* and *green*, respectively. **c** AKR from *M. tractuosa*. All-*trans*-retinal and NADPH are shown in *yellow* and *green*, respectively. **d** ALDH from *B. cereus*. All-*trans*-retinal and NAD^+ are shown in *yellow* and *green*, respectively

conditions. Increased production of retinoids has been achieved by increasing the solubility and stability of retinoids through the use of solvents and antioxidants. Retinoid-producing enzymes readily dissolved in water; however, in vitro retinoids are largely insoluble because of the hydrophobic properties of the alkene chain in retinoids (Duester 2000; During and Harrison 2004). Retinoids in an aqueous reaction solution are dissolved using an organic solvent. The solvents used for dissolving retinoids are ethanol (Belyaeva et al. 2008), dimethylformamide (Takahashi et al. 2009), and methanol (Hong et al. 2015). Methanol has been identified as the optimum solvent for retinol production by *M. tractuosa* AKR and *K. koreensis* ADH. To prevent the degradation of retinoids, antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), dithiothreitol (DTT), hydroquinone, and 2-mercaptoethanol are added in the reaction solutions (Su et al. 1999). Hydroquinone and BHT were found to be the optimal antioxidants for retinol production by *M. tractuosa* AKR and *K. koreensis* ADH (Hong et al. 2014a, 2015), while 2-mercaptoethanol is more suitable for retinoic acid produced by *Bacillus cereus* ALDH.

Retinoids produced by enzymes are summarized in Table 4. The production of retinoids by enzymes is increased by optimizing the substrate and enzyme concentrations (Kim et al. 2010; Hong et al. 2014a, 2015). The enzymatic production of retinal is highest in marine bacterium BCO. It produces 181 mg l^{-1} retinal from 350 mg l^{-1} β-carotene, with a conversion yield of 52 % (*w*/*w*) and a productivity of 9.1 mg l^{-1} h^{-1} (Kim et al. 2010). The enzymatic production of retinol is highest in *M. tractuosa* AKR, which produces 1090 mg l^{-1} all-*trans*-retinol from 1710 mg l^{-1} all-*trans*-retinal, with a conversion yield of 64 % (*w*/*w*) and a volumetric productivity of 818 mg l^{-1} h^{-1} (Hong et al. 2014a). *B. cereus* ALDH produces 63 mg l^{-1} retinoic acid from 120 mg l^{-1} retinal, with a conversion yield of 53 % (*w*/*w*) and a volumetric productivity of 15.8 mg l^{-1} h^{-1}.

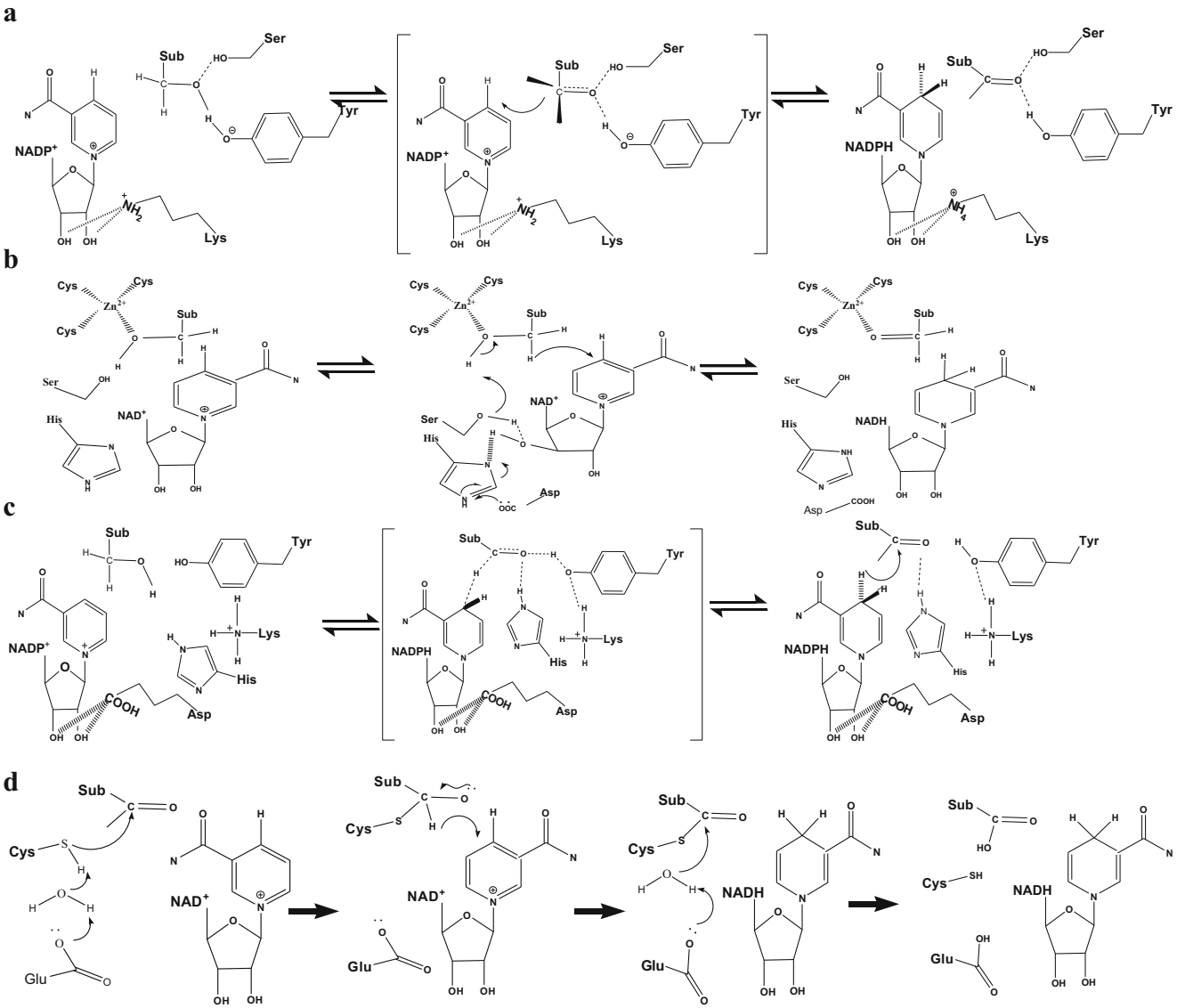

Fig. 3 Mechanisms of retinoid-converting enzymes. **a** RDH. **b** ADH. **c** AKR. **d** ALDH

Production of retinoids by metabolically engineered cells expressing BCO

The production of carotenoids has been carried out using metabolically engineered *E. coli*, which was prepared from the introduction of the foreign carotenoid synthesizing genes into non-carotenogenic *E. coli* (Misawa et al. 1990; Lee and Schmidt-Dannert 2002). *E. coli* has been identified as the most appropriate host for the production of retinoids (Lee et al. 2012; Jang et al. 2015) and carotenoids such as lycopene, β-carotene, canthaxanthin, zeaxanthin, and astaxanthin (Cunningham et al. 1993; Ruther et al. 1997; Ye et al. 2006).

Retinal has been produced from glycerol using metabolically engineered cells, which are prepared from the introduction of marine bacterium BCO gene into β-carotene-producing *E. coli* (Jang et al. 2011; Lee et al. 2012). The production of retinoids by metabolically engineered *E. coli* expressing BCO is summarized in Table 4. Metabolically engineered *E. coli* overexpressing ybbO (retinol dehydrogenase) gene in a flask produces 7 mg l^{-1} retinal, 78 mg l^{-1} retinol, and 24 mg l^{-1} retinoic acid after 72 h, with productivities of 0.1, 1.1, and 0.33 mg l^{-1} h^{-1}, respectively (Jang et al. 2015). However, genes related to the conversion of retinoids except for ybbO gene are not introduced yet into metabolically engineered cells. To enhance retinal production, temperature, pH, agitation speed, and surfactant concentration were optimized in a fed-batch culture of glycerol (Lee et al. 2012). Under optimal conditions, metabolically engineered *E. coli* produces 600 mg l^{-1} retinal for 33 h, with a productivity of 18 mg l^{-1} h^{-1}. This improved production of retinal is due to the culture using a bioreactor under optimized culture conditions.

Table 4 Production of retinoid by enzymes and metabolically engineered *E. coli*

Biocatalyst	Substrate (mg l^{-1})	Product (mg l^{-1})	Time (h)	Conversion yield (%, *w/w*)	Productivity (mg l^{-1} h^{-1})	References
K. koreensis ADH	All-*trans*-retinal (2200)	All-*trans*-retinol (600)	3.0	27	200	(Hong et al. 2015)
M. tractuosa AKR	All-*trans*-retinal (1710)	All- *trans*-retinol (1090)	2.7	64	818	(Hong et al. 2014a)
B. cereus ALDH	All-*trans*-retinal (120)	All- *trans*-retinoic acid (63)	4.0	53	15.8	This study
Marine bacterium BCO	β-Carotene (350)	All-*trans*-retinal (181)	20	52	9.1	(Kim et al. 2010)
Guinea pig BCO	β-Carotene (0.15)	All-*trans*-retinal (1.13×10^{-4})	2.0	0.08	5.65×10^{-5}	(Devery and Milborrow 1994)
Rat BCO	β-Carotene (2.67×10^{-5})	All-*trans*-retinal (4.66×10^{-6})	0.5	17	9.32×10^{-6}	(During et al. 1996)
Mouse BCO	β-Carotene (200)	All-*trans*-retinal (72)	2.0	36	4.8	(Kim et al. 2007)
Human BCO	β-Carotene (4.45×10^{-3})	All-*trans*-retinal (7.10×10^{-5})	1.0	1.6	7.10×10^{-5}	(Lindqvist and Andersson 2002)
Human BCO	β-Carotene (200)	All-*trans*-retinal (98)	16	49	6.1	(Kim et al. 2008)
Metabolic engineered *E. coli*	Glycerol (140,000)	All-*trans*-retinal (600)	33	NR	18	(Lee et al. 2012)
Metabolic engineered *E. coli*	Glycerol (20,000)	All-*trans*-retinal (67)	72	0.34	0.93	(Jang et al. 2011)
Metabolic engineered *E. coli*	Glycerol (20,000)	All-*trans*-retinol (54)	72	0.27	0.75	(Jang et al. 2011)
Metabolic engineered *E. coli*	Glycerol (20,000)	All-*trans*-retinoic acid (15)	72	0.08	0.21	(Jang et al. 2011)
Metabolic engineered-ybbO *E. coli*	Glycerol (20,000)	All-*trans*-retinal (7)	72	0.04	0.1	(Jang et al. 2015)
Metabolic engineered-ybbO *E. coli*	Glycerol (20,000)	All-*trans*-retinol (78)	72	0.39	1.1	(Jang et al. 2015)
Metabolic engineered-ybbO *E. coli*	Glycerol (20,000)	All-*trans*-retinoic acid (24)	72	0.12	0.33	(Jang et al. 2015)

NR not reported, *BCO* β-carotene 15,15′-oxygenase

Phylogenetic features

We used 34 genes, which involved in the retinoid conversion, to construct a phylogenetic analysis. These genes consisted of 27 genes from mammals and 8 genes from bacteria. The phylogenetic tree of retinal-converting enzymes divides into the five groups such as RDHs, ADHs, AKRs, ARATs, and ALDHs (Fig. 4). ADHs from *K. koreensis* and *Aeromonas hydrophila* are well grouped with mammals. In contrast, AKRs from *Croceibacter atlanticus* and *M. tractuosa* and RDHs from *Myxococcus xanthus* and *Alicyclobacillus acidocaldarius* form independently own clades, distinguishing from other mammalian ADHs, AKRs, and RDHs, respectively. Interestingly, *B. cereus* ALDH in the phylogenetic tree is found within AKRs from mammals.

Further research

Bacterial sources of retinoid-converting enzymes

To date, the biochemical properties and structures of many animal retinoid-converting enzymes have been characterized (Table 2). However, bacterial enzymes have some advantages over their animal counterparts. Bacterial enzymes exhibit greater stability and are easier to express in *E. coli* than animal enzymes. Large quantities of bacteria can be easily cultured in a short time, and the manipulation of bacterial genes in *E. coli* host is generally easier to accomplish than that animal genes (Anbu et al. 2013). There is a greater potential for the use of bacterial enzymes in industrial applications than animal enzymes, and we propose that more retinoid-converting enzymes

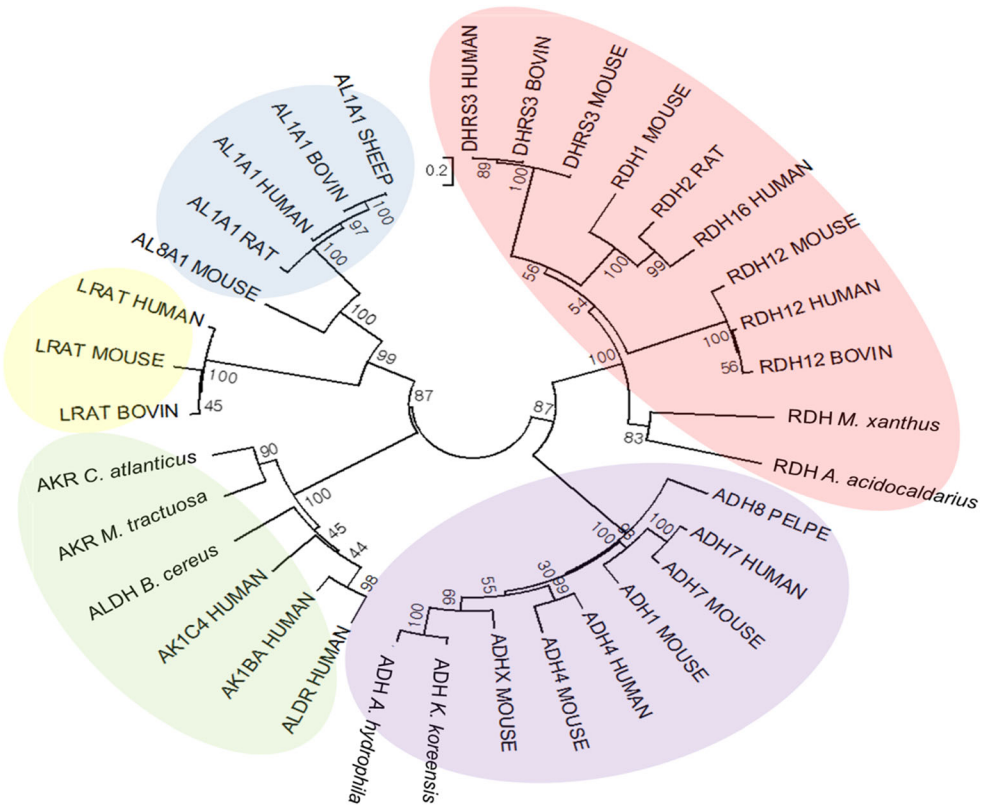

Fig. 4 Phylogenetic tree analysis of RDH, ADH, AKR, and ARAT amino acid sequences from mammals and bacteria. Amino acid sequences were aligned with Clustal Omega program (UniProt) and phylogram were constructed with MEGA5 (Tamura et al. 2013). The construction of the phylogenetic tree was made using the neighbor-joining method (Saitou and Nei 1987). The optimal tree was represented with the sum of branch length = 14.12866066. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method (Zuckerkandl and Pauling 1965) and are in the units of the number of amino acid substitutions per site. All ambiguous positions were removed for each sequence pair. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013). RDHs (*red colored*), ADHs (*purple colored*), AKRs (*green colored*), ARATs (*yellow colored*), and ALDHs (*blue colored*). Accession numbers; RDH12 human: Q96NR8; RDH12 mouse: Q8BYK4; RDH12 bovine: P59837; DHRS3 human: O75911; DHRS3 mouse: O88876; DHRS3 bovine: O77769; RDH2 rat: P50170; RDH1 mouse: O55240; RDH16 human: P75452; ADH1 mouse: P00329; ADH4 human: P08319; ADH4 mouse: Q9QYY9; ADHX mouse: P28474; ADH7 human: P40394; ADH7 mouse: Q64437; ADH8 pelpe (frog): O57380; ALDR human: P15121; AK1BA human: O60218; AK1C4 human: P17516; AL1A1 human: P00352;AL1A1 rat: P51647; AL8A1 mouse: Q8BH00; AL1A1 bovine: P48644; AL1A1 sheep: P51977; LRAT bovine: Q9BGL2; LRAT human: O95237; LRAT mouse: Q9JI60; *M. xanthus* RDH: Q1D4P2; *A. acidocaldarius* RDH: C8WQV1; *K. koreensis* ADH: C7R702; *A. hydrophila* ADH: A0KES6; *M. tractuosa* AKR: E4TQF9; *C. atlanticus* AKR: A3U9Y0; *B. cereus* AKR: C2MV60; *B. cereus* ALDH: C2N217

should be obtained from bacterial sources. Many bacteria contain alcohol dehydrogenases and aldehyde dehydrogenases for the detoxification of aldehydes. However, the confirmation for the activities of these enzymes towards retinoids remains unclear. After identifying these activities, the biochemical properties of the bacterial enzymes should be characterized.

ADH from *K. koreensis* (Hong et al. 2015) and AKR from *M. tractuosa* (Hong et al. 2014a) have been previously reported as bacterial retinol-converting enzymes. RDHs from *M. xanthus* and *A. acidocaldarius*, ADH from *A. hydrophilia*, and AKRs from *B. cereus* and *C. atlanticus* convert retinal to retinol, while ALDH from *B. cereus* converts retinal to retinoic acid (Table 2). We propose that in the bacterial RDH, ADH, AKR, and ALDH, ARAT proteins can catalyze the conversion of retinal to retinol or retinoic acid and that the bacterial ARAT protein can catalyze the conversion of retinol or retinyl ester, based on sequence alignment and the comparison of active-site residues (Table 5). The *rdh*, *adh*, *akr*, *aldh*, and *arat* genes of these bacteria should be cloned, and the expressed proteins should be used for the production of retinol, retinoic acid, and retinyl ester. Moreover, the mutations in the amino acids on or near the active site should be attempted to increase the specific activities of these retinoid-converting enzymes.

Production of retinoids using metabolically engineered cells harboring bacterial retinoid-converting enzymes

The construction of a library for bacterial RDHs, ADHs, AKRs, ALDHs, and ARATs would assist in improving the production of retinoids by metabolically engineered cells. An efficient retinoid-converting enzyme can be selected from the library. Gene encoding the effective retinoid-converting enzymes, such as *rdh*, *adh*, *akr*, *aldh*, or *arat*, can be introduced into retinal-producing metabolically engineered *E. coli*, thereby resulting in the production of retinol, retinoic acid, and retinyl ester. The culture conditions, such as medium composition, temperature, pH, and culture time of retinoid-producing metabolically engineered *E. coli*, should be optimized for the enhanced production of retinoids. To prevent the transformation of retinoids into ineffective forms via hydrolysis, genes involved in the degradation of retinoids should be removed. Retinoids are known to be unstable in the presence of oxygen and when exposed to light (O'Flaherty et al. 1997; Han et al. 2003). The low stability of retinoids has limited their production in large quantities under aqueous conditions. To overcome this limitation, enzyme reaction systems, such as two-phase and co-solvent systems, need to be developed. The degradation of retinoids can also be prevented by supplementing culture systems with additives such as fatty acids, antioxidants, and detergents.

In conclusion, we have presented a review of the biochemical properties, active sites, and reaction mechanisms of enzymes involved in the production of retinoids. In addition, we have described the production of retinoids using retinoid-producing enzymes and metabolically engineered cells. We focused on bacterial enzymes because they exhibited greater stability than their animal counterparts; it is also much easier and more cost-effective to produce recombinant bacterial proteins than recombinant animal proteins. Thus, bacterial uncharacterized proteins are suggested as retinoid-converting

Table 5 Candidate bacterial retinoid-converting enzymes

Division	Organism	Family	Enzyme name	Gene name	Protein acc. no
Bacteria	*Hyalangium minutum*	RDH	3-Oxoacyl-[acyl-carrier protein] reductase	DB31_1681	KFE64663.1
	Cystobacter violaceus		Short-chain dehydrogenase	Q664_30270	KFA90250.1
	Myxococcus stipitatus		Short-chain dehydrogenase	MYSTI_03749	AGC45055.1
	Enhygromyxa salina		3-Oxoacyl-[acyl-carrier protein] reductase	DB30_6693	KFE74757.1
	Sulfolobus solfataricus	ADH	Alcohol dehydrogenase 8	Adh8	AAK42630.1
	Haloarcula marismortui		Alcohol dehydrogenase 8	Adh8	AAV44813.1
	Halobacterium salinarum		Alcohol dehydrogenase	Adh3	AAG19432.1.
	Natronomonas moolapensis	AKR	Aldo-keto reductase family	Nmlp_1330	CCQ35538.1
	Halophilic archaeon 1		Aldo-keto reductase family	Halar_2150	AEN05831.1
	Halorubrum lipolyticum		Aldo-keto reductase	C469_06194	EMA61757.1
	Alicyclobacillus acidocaldarius	ALDH	Aldehyde dehydrogenase	Aaci_2504	ACV59510.1
	Viridibacillus arenosi		Aldehyde dehydrogenase	C176_02129	ETT88046.1
	Bacillus thuringiensis		Betaine aldehyde dehydrogenase	BTCBT_003997	ERI00023.1
	Aeromonas sp.	ARAT	Contig_76, whole genome shotgun sequence	RW26_18935	KIQ78046.1
	Campylobacter concisus		NC domain family	CCC13826_1046	EAT97823.1
	Pontibacillus halophilus		Uncharacterized protein	N781_14970	KGX92625.1

enzymes, and the production of retinoids using metabolically engineered cells harboring bacterial retinoid-converting enzymes is proposed as a feasible method. However, these biotechnological methods are still in the early stage. In the future, the biotechnological methods will be able to use the industrial production of retinoids because biotechnological methods rapidly develop and replace chemical methods.

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Conflict of interest The authors declare that they have no competing interests.

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