

Biotechnological production of polyamines by Bacteria: recent achievements and future perspectives

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Abstract In Bacteria, the pathways of polyamine biosynthesis start with the amino acids L-lysine, L-ornithine, L-arginine, or L-aspartic acid. Some of these polyamines are of special interest due to their use in the production of engineering plastics (e.g., polyamides) or as curing agents in polymer applications. At present, the polyamines for industrial use are mainly synthesized on chemical routes. However, since a commercial market for polyamines as well as an industry for the fermentative production of amino acid exist, and since bacterial strains overproducing the polyamine precursors L-lysine, L-ornithine, and L-arginine are known, it was envisioned to engineer these amino acid-producing strains for polyamine production. Only recently, researchers have investigated the potential of amino acid-producing strains of *Corynebacterium glutamicum* and *Escherichia coli* for polyamine production. This mini-review illustrates the current knowledge of polyamine metabolism in Bacteria, including anabolism, catabolism, uptake, and excretion. The recent advances in engineering the industrial model bacteria *C. glutamicum* and *E. coli* for efficient production of the most promising polyamines, putrescine (1,4-diaminobutane), and cadaverine (1,5-diaminopentane), are discussed in more detail.

Keywords *Corynebacterium glutamicum* · *Escherichia coli* · Polyamines · Polyamides · Polymer · Cadaverine · Putrescine · 1,5-diaminopentane · 1,4-diaminobutane

Introduction

Alkaline organic compounds with an aliphatic, saturated carbon backbone, at least two primary amino groups, and a varying number of secondary amino groups are referred to as polyamines (Fig. 1). They are present in virtually every living cell and it has been shown that they can modulate diverse cellular processes, including transcription and translation, possibly due to their positive charge distribution at physiological pH (Wallace et al. 2003). However, a detailed understanding of the mechanisms remains unclear. Various additional functions of bacterial polyamines have also been uncovered: they are part of outer membranes of Gram-negative bacteria (Takatsuka and Kamio 2004), are involved in the biosynthesis of siderophores (Brickman and Armstrong 1996), take part in acid resistance (Foster 2004), protect from oxygen toxicity (Jung et al. 2003), play a role in signaling for cellular differentiation (Sturgill and Rather 2004), and are essential for plaque biofilm formation (Patel et al. 2006). It was assumed that polyamines are essential for life and this might still be true for several species, especially eukaryotes. However, in a recent publication, a polyamine-free *Escherichia coli* strain, which was able to grow under the selected conditions, has been described (Chattopadhyay et al. 2009).

Depending on the species, the relative intracellular concentrations of the different polyamines may vary, and they can reach high concentrations, up to the millimolar range (Miyamoto et al. 1993). The most common polyamines in *Bacteria* and *Archaea* are putrescine, a diamine also named 1,4-diaminobutane, and the triamine spermidine, whereas cadaverine, a diamine also named 1,5-diaminopentane, is much less abundant. Although the tetraamine spermine has been detected in *Bacteria* and *Archaea* (Hamana and Matsuzaki 1992), spermine synthase

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A) Linear Polyamines			B) Branched Polyamines		
Group	Name*	Structure	Group	Name*	Structure
Diamines	1,3-Diaminopropane		Triamines	N ⁴ -Aminopropyl norspermidine	
	Putrescine				N ⁴ -Aminopropyl spermidine
	Cadaverine			Tetraamines	
Triamines	Spermidine				N ⁴ -Bis(aminopropyl) spermidine
	Homospermidine		N ⁴ -Bis(aminopropyl) spermidine		
	Norspermidine				Tetraamines
	Aminopropylcadaverine		Tetraamines	N ⁴ -Bis(aminopropyl) spermidine	
Tetraamines	Thermine				Tetraamines
	Spermine		Tetraamines	N ⁴ -Bis(aminopropyl) spermidine	
	Thermospermine				Tetraamines
	Homospermine		Pentaamines	N ⁴ -Bis(aminopropyl) norspermidine	
Pentaamines	Caldopentamine				Pentaamines
	Thermopentamine		Pentaamines	N ⁴ -Bis(aminopropyl) spermidine	
	Homocaldopentamine				Hexaamines
Hexaamines	Caldohexamine		Hexaamines	N ⁴ -Bis(aminopropyl) spermidine	
	Thermohexamine				Hexaamines
	Homocaldohexamine		Hexaamines	N ⁴ -Bis(aminopropyl) spermidine	
	Homothermohexamine				Hexaamines

Fig. 1 Common and unique polyamines detected in Bacteria and Archaea. All polyamines are shown, despite the fact that the specific biosynthetic pathways might be unknown at present. *Asterisk* systematic names are used only if a trivial name is unknown

(EC 2.5.1.22) activity has yet to be proved. It could be possible that spermine is synthesized in these species by a non-specific aminopropyltransferase. In addition to the above-mentioned polyamines, the pathways for the biosynthesis of 1,3-diaminopropane, norspermidine, homospermidine, and thermine are known in some *Bacteria* and *Archaea*. This set of polyamines is extended by a number of uncommon longer- or branched-chain polyamines, which were found in extremophiles (Fig. 1a, b) and which seem to play an essential role for growth under such extreme conditions (Oshima 2007). The branched-chain polyamines (Fig. 1b) are no polyamines *sensu stricto* as they contain tertiary and quaternary amino groups. However, except for caldopentamine, the biosynthetic pathways for these uncommon longer- or branched-chain polyamines have not yet been investigated in detail (Knott 2009; Oshima, 2010).

Polyamines are applied in a wide variety of commercial applications due to their unique combination of reactivity, basicity, and surface activity. With a few exceptions, they are used predominantly as intermediates in the production of functional products (e.g., polyamides/epoxy curing, fungicide, anthelmintics/pharmaceuticals, petroleum production, oil and fuel additives, paper resins, chelating agents, fabric softeners/surfactants, bleach activator, asphalt chemicals) (Kroschwitz and Seidel 2004). The main commercial interest in biogenic polyamines is their use in the polymer industry.

Diamines can be used as monomers for the production of polyamides, whereas higher amines can be applied as curing agents in epoxy-resins.

Polyamides are synthetic polymers containing amid-linker, which are formed by a condensation reaction between an amino and a carboxy moiety. The polyamide monomers can either be lactams comprising an intramolecular cyclic amide (AB-type, e.g. Nylon-6, Nylon-11) or they are monofunctional comprising either amino or carboxy moieties (AA/BB-type, e.g. Nylon-4,6). The properties of polyamides depend on the chosen monomer-type, with influences of, e.g., the aliphatic or aromatic carbon backbone or the length of the backbone. In principle, a vast of number of combinations is possible. Today, the only example of an industrial polyamide containing a biogenic diamine, which can also be synthesized by *Bacteria*, is nylon-4,6. This polyamide is produced from putrescine and adipic acid (hexanedioic acid) and is marketed, e.g., under the trademark Stanyl by DSM, Netherlands.

The total market for plastics grew in the last six decades with an average of 9% per year and the polyamide fraction grew with 4.5% per year in the last years, reaching a predicted production volume of 2.4 million tons per year in 2007 (Platt 2003). The market share of Stanyl and other special polyamides is about 2% of this market. At present,

the production of monomers for use in the polyamide industry is mainly based on chemical routes, with few exceptions like 1,10-decamethylenediamine which is isolated from castor oil and used for production of PA-10,10 (e.g., Vestamid TerraDS by Evonik Degussa, Germany), but a drive towards low-carbon footprints of materials and applications and towards the development of sustainable processes might further promote biotechnological polyamine production.

In Bacteria, the pathways of polyamine biosynthesis start with the amino acids L-lysine, L-ornithine, L-arginine, or L-aspartic acid. Since a million-ton-scale industry for the fermentative production of amino acid thrives and strains overproducing L-lysine, L-ornithine, and L-arginine are available, it is reasonable to engineer these amino acid-producing bacteria for polyamine production. Biotechnological production of polyamines, which necessitates tolerance to high product concentrations, has been not investigated until recently. This might be due to toxicity of polyamines or their derivatives if they accumulate in the producer cells (Limsuwun and Jones 2000). However, the toxic effects seem to be relevant for few polyamines in general, as recent publications concerning polyamine production by bacteria show very promising results (Table 1).

The focus of this mini-review is on the biotechnological production of polyamines by means of *Bacteria*. For information on polyamines in eukaryotes, we refer to other reviews (Seiler 1990; Seiler 2004). In the following, a survey on polyamine metabolism in *Bacteria* and *Archaea* will be given, and recent metabolic engineering approaches for production of diamines by *E. coli* and *C. glutamicum* will be discussed.

Polyamine metabolism in Bacteria and Archaea

This paragraph provides an overview on anabolic and catabolic polyamine pathways investigated so far in *Bacteria* and *Archaea*. The currently known pathways are summarized in Fig. 2.

Anabolic pathways

Cadaverine The formation of cadaverine occurs through the decarboxylation of L-lysine (Fig. 2, reaction 1), and this polyamine is only present in minor amounts in *E. coli* under normal growth conditions (Tabor and Tabor 1985). In *E. coli*, two isozymes catalyze L-lysine decarboxylation: the acid-inducible CadA (Sabo et al. 1974) and the constitutive LdcC (Kikuchi et al. 1997). The production of CadA is induced at low pH and plays a role in the acid resistance system of *E. coli*. As L-lysine decarboxylation consumes

one proton, the intracellular pH increases, which contributes to pH homeostasis (Foster 2004).

Putrescine Putrescine can be formed in vivo via the ODC or ADC pathway, named after the enzymes ornithine decarboxylase (ODC) and arginine decarboxylase (ADC), respectively, which catalyze the initial reaction of the pathways. As described above for L-lysine decarboxylation, the ODC and ADC pathways also contribute to acid resistance in *E. coli* by proton-consuming decarboxylation of L-ornithine and L-arginine, respectively (Foster 2004). Two isozymes for both decarboxylation reactions are encoded by the genome of *E. coli*: the acid-inducible decarboxylases SpeF (L-ornithine-specific) and AdiA (L-arginine-specific) as well as the constitutive decarboxylases SpeC (L-ornithine-specific) and SpeA (L-arginine-specific) (Applebaum et al. 1975, Blethen et al. 1968, Applebaum et al. 1977, Wu and Morris 1973). The acid-inducible decarboxylases are sometimes referred to as degradative enzymes, while the constitutive decarboxylases are named biosynthetic enzymes. In the ODC pathway, L-ornithine is directly converted to putrescine by ornithine decarboxylase (Fig. 2, reaction 2). By contrast, decarboxylation of L-arginine represents only the first step of the ADC pathway (Fig. 2, reaction 4). The product of L-arginine decarboxylation, agmatine, is converted in one of three species-specific reactions. In *E. coli*, agmatine is directly hydrolyzed to putrescine and urea by agmatinase SpeB (Fig. 2, reaction 5) (Sathishchandran and Boyle 1986). In some bacterial species, agmatine is converted to putrescine in a two-step reaction (Fig. 2, reactions 6+7): hydrolytic deimination of agmatine to *N*-carbamoylputrescine by agmatine deiminase AguA followed by hydrolysis of the carbamoyl group to yield ammonia, carbon dioxide, and putrescine in a reaction catalyzed by *N*-carbamoylputrescine amidohydrolase AguB (Nakada and Itoh 2003). A variation of the last step is found in *Enterococcus faecalis*, where putrescine transcarbamylase releases carbamoylphosphate from *N*-carbamoylputrescine and inorganic phosphate to yield putrescine (Fig. 2, reaction 8) (Wargnies et al. 1979).

Spermidine and norspermidine The common pathway for spermidine formation is the aminopropylation of putrescine with decarboxylated S-adenosylmethionine (dcSAM) as the donor molecule (Fig. 2, reaction 11) (Bowman et al. 1973), but recently, another pathway starting from agmatine was discovered in extremophiles (Ohnuma et al. 2005; Morimoto et al. 2010). In these bacteria, agmatine aminopropyltransferase uses dcSAM as a donor to produce aminopropylagmatine which is subsequently hydrolyzed to spermidine and urea by aminopropylagmatine ureohydrolase (Fig. 2, reactions 9+10). A third pathway for spermidine formation is known (Tait 1976), and the relevant enzymes have been

Table 1 Characteristics of putrescine and cadaverine production by different *C. glutamicum* and *E. coli* strains

Polyamine	Substrate	Organism	Cultivation method	c [g l ⁻¹]	$Y_{(p/S)}$ [g g ⁻¹]	$Y_{(p/X)}$ [g g ⁻¹]	q_p [g l ⁻¹ h ⁻¹]	Reference
Putrescine	Glucose	<i>E. coli</i>	Fermentor (fed-batch)	5.1	n.d.	0.40	n.d.	(Eppelmann et al. 2006)
	Glucose	<i>E. coli</i>	Fermentor (fed-batch)	24.2	n.d.	1.16 ^a	0.75	(Qian et al. 2009)
	Glucose	<i>C. glutamicum</i>	Shake-flask	6.0	0.12	0.65	0.10	(Schneider and Wendisch 2010)
	Glucose	<i>C. glutamicum</i>	Fermentor (fed-batch)	18.6	0.16	1.01	0.55	(Schneider et al., unpublished)
Cadaverine	Lysine	<i>E. coli</i>	Fermentor (fed-batch)	69.0	–	–	–	(Nishi et al. 2007)
	Glucose	<i>E. coli</i>	Fermentor (fed-batch)	9.6	0.12	0.59 ^a	0.32	(Qian et al. 2011)
	Glucose	<i>C. glutamicum</i>	Fermentor (batch)	2.6	0.05 ^a	n.d.	0.14 ^a	(Mimitsuka et al. 2007)
	Glucose	<i>C. glutamicum</i>	Shake-flask	3.4	n.d.	n.d.	n.d.	(Verseck et al. 2008)
	Glucose	<i>C. glutamicum</i>	Fermentor (batch)	5.0	0.09 ^a	n.d.	0.24 ^a	(Tateno et al. 2008)
	Starch	<i>C. glutamicum</i>	Fermentor (batch)	2.4	0.05 ^a	n.d.	0.11 ^a	(Tateno et al. 2008)
	Glucose	<i>C. glutamicum</i> ^b	Shake-flask	1.7 ^a	0.17 ^a	0.49 ^a	n.d.	(Kind et al. 2010b)
	Glucose	<i>C. glutamicum</i> ^b	Shake-flask	1.1 ^a	0.11 ^a	0.31 ^a	n.d.	(Kind et al. 2010b)
	Glucose	<i>C. glutamicum</i>	Shake-flask	1.3 ^a	0.13 ^a	0.38 ^a	n.d.	(Kind et al. 2010a)
	Glucose	<i>C. glutamicum</i>	Fermentor (fed-batch)	72.0	n.d.	n.d.	0.90 ^a	(Völkert et al. 2010)
	Xylose	<i>C. glutamicum</i>	Shake-flask	1.4 ^a	0.11 ^a	0.60 ^a	0.04	(Buschke et al. 2011)
	Hemicellulose hydrolysate	<i>C. glutamicum</i>	Shake-flask	2.0 ^a	n.d.	n.d.	0.07 ^a	(Buschke et al. 2011)

n.d. not determined

^a Indicates numbers calculated based on values in the relevant reference

^b Same strain but different media supplementation

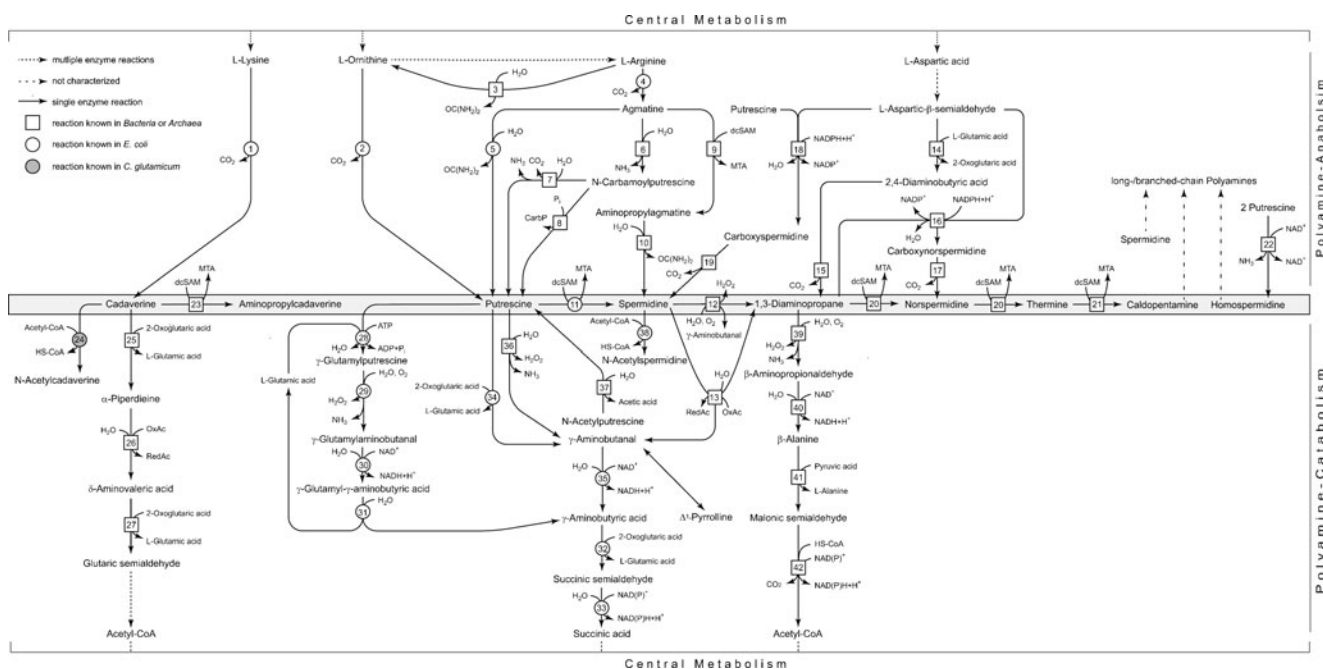


Fig. 2 Schematic representation of known anabolic and catabolic polyamine pathways in Bacteria and Archaea. Enzymatic side reactions which were shown only in vitro are not included. *dcSAM* decarboxylated *S*-adenosylmethionine, *MTA* methylthioadenosine, *OxAc* oxidized electron acceptor, *RedAc* reduced electron acceptor, *1* lysine decarboxylase (EC 4.1.1.18), *2* ornithine decarboxylase (EC 4.1.1.17), *3* arginase (EC 3.5.3.1), *4* arginine decarboxylase (EC 4.1.1.19), *5* agmatinase (EC 3.5.3.11), *6* agmatine deiminase (EC 3.5.3.12), *7* *N*-carbamoylputrescine amidohydrolase (EC 3.5.1.53), *8* putrescine transcarbamylase (EC 2.1.3.6), *9* agmatine aminopropyltransferase (EC 2.5.1.-), *10* aminopropylagmatine ureohydrolase (EC 3.5.3.-), *11* spermidine synthase (EC 2.5.1.16), *12* spermidine oxidase (EC 1.5.3.B4), *13* spermidine dehydrogenase (EC 1.5.99.6), *14* diaminobutyric acid transaminase (EC 2.6.1.76), *15* diaminobutyric acid decarboxylase (EC 4.1.1.86), *16* carboxy(nor)spermidine dehydrogenase (EC unknown), *17* carboxy(nor)spermidine decarboxylase (EC 4.1.1.-), *18* carboxy(nor)spermidine dehydrogenase (EC unknown), *19* carboxy(nor)spermidine decarboxylase (EC 4.1.1.-), *20* aminopropyl

transferase (EC 2.5.1.-), *21* aminopropyl transferase (EC 2.5.1.-), *22* homospermidine synthase (EC 2.5.1.44), *23* cadaverine aminopropyltransferase (EC 2.5.1.-), *24* diamine acetyltransferase (EC 2.3.1.57), *25* cadaverine aminotransferase (EC 2.6.1.-), *26* α -piperidine dehydrogenase (EC 1.5.-), *27* δ -aminovaleric acid aminotransferase (EC 2.6.1.-), *28* γ -glutamylputrescine synthase (EC 6.3.1.11), *29* γ -glutamylputrescine oxidase (EC 1.4.3.-), *30* γ -glutamyl- γ -aminobutyraldehyde dehydrogenase (EC 1.2.1.-), *31* γ -glutamyl- γ -aminobutyrate hydrolase (EC 3.5.1.94), *32* γ -aminobutyric acid aminotransferase (EC 2.6.1.19), *33* succinic acid semialdehyde dehydrogenase (EC 1.2.1.16), *34* putrescine aminotransferase (EC 2.6.1.82), *35* aminobutyraldehyde dehydrogenase (EC 1.2.1.19), *36* putrescine oxidase (EC 1.4.3.10), *37* *N*-acetylputrescine decetylase (EC 3.5.1.62), *38* spermidine acetyltransferase (EC 2.3.1.57), *39* primary amine oxidase (EC 1.4.3.21), *40* aldehyde dehydrogenase (EC 1.2.1.3), *41* β -alanine transaminase (EC 2.6.1.19), *42* malonic acid semialdehyde decarboxylase (EC 1.2.1.18)

characterized in members of the genus *Vibrio* (Fig. 2, reactions 18+19). In *Vibrio*, L-aspartic-semialdehyde, an intermediate in L-lysine biosynthesis, and putrescine are reductively condensed by carboxy(nor)spermidine dehydrogenase (CANSDH) (Nakao et al. 1991). The reaction product, carboxyspermidine, is then decarboxylated to spermidine by carboxy(nor)spermidine decarboxylase (CANSDC) (Nakao et al. 1990).

Vibrio species also synthesize norspermidine from L-aspartic-semialdehyde which is condensed with 1,3-diaminopropane (Fig. 2, reaction 14–17) (Yamamoto et al. 1986). Amination of L-aspartic-semialdehyde to 2,4-diaminobutyric acid and subsequent decarboxylation yields 1,3-diaminopropane, which is condensed with another molecule of L-aspartic-semialdehyde by CANSDH to yield carboxynorspermidine. Carboxynorspermidine is decarboxylated to norspermidine by

CANSDC. These reactions were investigated in detail in *Vibrio cholerae* but homologs of the key enzymes were found to be widespread among *Bacteria* (Lee et al. 2009). As alternative to the *Vibrio* pathway, aminopropyl transferase activity which uses *dcSAM* to synthesize norspermidine from 1,3-diaminopropane was detected in *Clostridium thermohydrosulfuricum* (Fig. 2, reaction 20) (Paulin et al. 1983).

Other polyamines Besides the reactions involved in the synthesis of the polyamines mentioned above, enzymes catalyzing formation of homospermidine, thermine, and caldopentamine have been characterized. Homospermidine can be formed by condensation of two molecules of putrescine (Fig. 2, reaction 22). This reaction is catalyzed by a single enzyme, which oxidatively deaminates putrescine to yield ammonia, aminobutyraldehyde, and NADH.

The latter two remain enzyme-bound and the enzyme-bound aminobutyraldehyde forms an imine with a second molecule of putrescine. This enzyme-bound imine is reduced by the enzyme-bound NADH to yield homospermidine (Tait 1979). It was shown that aminopropyl transferase from *Pyrobaculum aerophilum* has the highest activity towards thermine formation with the substrate norspermidine and the aminopropyl donor dcSAM (Fig. 2, reaction 20) (Knott 2009). The aminopropyl transferase from *C. thermohydrosulfuricum* also accepts norspermidine as a substrate in addition to 1,3-diaminopropane which leads to thermine formation (Fig. 2, reaction 20) (Paulin et al. 1983). In *Hyperthermus butylicus*, a polyamine synthase is present which catalyzes the formation of caldopentamine from thermine and dcSAM (Knott 2009). This is at present the only known enzyme for synthesis of long-chain polyamine synthesis beyond the tetraamines.

Catabolic pathways

In the view of metabolic engineering, an ideal production host cannot degrade or utilize the product. However, if the desired product is consumed rather than excreted and the overall yield of the process decreases, the corresponding pathways become an important engineering target. Therefore, knowledge about these pathways is required and in the following, the catabolism of cadaverine, putrescine, and spermidine is described. Degradation or utilization of the other known bacterial or archaeal polyamines has not yet been investigated.

Cadaverine Several bacteria are known to degrade L-lysine through decarboxylation, which leads to cadaverine as the first intermediate. This pathway has been studied in detail in members of the genus *Pseudomonas* (Fothergill and Guest 1977; Hofle 1984; Madduri et al. 1989). Cadaverine is then further metabolized by transamination to α -piperidine and oxidized to δ -aminovaleric acid (AMV). This intermediate is eponymous for the so-called AMV pathway that leads to acetyl-CoA (Revelles et al. 2005) (Fig. 2, reactions 25–27). It is also known that cadaverine is a substrate for aminopropylation and acetylation in some species (Fig. 2, reactions 23 and 24) (Cacciapuoti et al. 2007; Kind et al. 2010a). The utilization or degradation of cadaverine has not been observed for *E. coli* and *C. glutamicum*.

Putrescine Besides dcSAM-dependent conversion to spermidine, putrescine can be utilized as a carbon source by two pathways, which have been investigated in *E. coli*. Both pathways lead to GABA (γ -aminobutyric acid), which is then deaminated and oxidized to the tricarboxylic acid

cycle intermediate succinic acid (Fig. 2, reactions 32+33). In *E. coli*, the latter two enzymes are encoded by the constitutive *gabT/gabD* and by the putrescine inducible *puuE/lyneI* (Kurihara et al. 2010).

The initial step of the first pathway for putrescine degradation, the Puu pathway, is the glutamylation of putrescine to γ -glutamylputrescine. In three subsequent reactions, γ -glutamylputrescine is oxidized to GABA (Kurihara et al. 2005) (Fig. 2, reactions 28–31). The second pathway, the YgiG–YdcW pathway, involves the transamination of putrescine to γ -aminobutanal followed by oxidization to GABA (Shaibe et al. 1985) (Fig. 2, reactions 34+35). Besides transamination of putrescine to γ -aminobutanal, putrescine can also be reductively deaminated by putrescine oxidase in a reaction yielding hydrogen peroxide (van Hellemond et al. 2008) (Fig. 2, reaction 36). Specific deacetylation of *N*-acetyl-putrescine was detected in *Bacteria* (Fig. 2, reaction 37) (Suzuki et al. 1986), even though acetylation of putrescine has not yet been described in *Bacteria* or *Archaea*.

Spermidine Spermidine is catabolized via 1,3-diaminopropane and γ -aminobutanal either by spermidine oxidase or by spermidine dehydrogenase (Bachrach 1962b; Hisano et al. 1990) (Fig. 2, reactions 12+13). The further degradation of γ -aminobutanal to succinic acid has already been described above for putrescine degradation. 1,3-Diaminopropane can be further metabolized via β -alanine and malonic semialdehyde to acetyl-CoA (Bachrach 1962a) (Fig. 2, reactions 39–42). The utilization of spermidine has neither been shown for *E. coli* nor for *C. glutamicum*, but the acetylation of spermidine has been described for *E. coli* (Fig. 2, reaction 38) (Limsuwun and Jones 2000; Matsui et al. 1982).

Transport processes across the cell membrane

In the following paragraph, transport capabilities of bacterial species concerning amino acids and polyamines are described with respect to metabolic engineering of polyamine production hosts. The enhancement of product formation by modulation of transport processes across the cell membrane is well known (Lee et al. 2007; Gunji and Yasueda 2006). Hence, genes encoding proteins for product export might be good targets for overexpression, whereas genes encoding proteins facilitating re-uptake of the secreted product or the efflux of precursors might be deletion targets. Overexpression of transporter genes may be very challenging as, e.g. a wide substrate range could cause a drastic decrease in the overall strain fitness and/or the membrane integrity might be compromised. Also, the inhibition of excretion capabilities can drastically influence the cell metabolism.

As a good review article on polyamine transport in *E. coli* was published by Igarashi and Kashiwagi (2010), only a brief overview is given here and some extensions of known transport processes to other bacteria are made. To our knowledge, for bacteria, only information on transport of the polyamines cadaverine, putrescine, and spermidine has been published. In Fig. 3, biochemically characterized or observed transport processes of polyamines, derivatives, and precursors in bacterial species are outlined.

Cadaverine The uptake and excretion of cadaverine in *E. coli* is mediated by CadB, a protein of the amino acid-polyamine-organocation (APC) family. Members of this family function as solute:cation symporter and/or as solute:solute antiporter. CadB is responsible for cadaverine uptake at neutral pH by proton-linked symport while at acidic pH, CadB mediates excretion of cadaverine in antiport with L-lysine (Soksawatmaekhin et al. 2004). Accumulation of N-acetylated cadaverine in the fermentation broth was observed for *C. glutamicum*, but the transport system is unknown (Kind et al. 2010b; Kind et al. 2010a). In *E. coli*, export of the cadaverine precursor L-lysine involves YahN (Park and Lee 2010), and L-lysine uptake may be mediated by LysP (Steffes et al. 1992) or ArgT-HisJQMP (Rosen 1971). YahN is a member of the resistance to homoserine/threonine (RhtB) family and catalyzes export of L-lysine coupled to proton import. Uptake of L-lysine into the *E. coli* cell is catalyzed by APC-type permease LysP or by the ATP-binding cassette (ABC) transporter ArgT-HisJQMP.

In *C. glutamicum*, LysI is the uptake system for L-lysine (Seep-Feldhaus et al. 1991) and LysE mediates the export. LysE from *C. glutamicum* was the first amino acid export system discovered in *Bacteria* (Vrljic et al. 1996) and is eponymous for the LysE superfamily of export proteins (Vrljic et al. 1999). Besides L-lysine, LysE also catalyzes export of D-lysine (Stäbler et al. 2011) and L-arginine

(Bellmann et al. 2001). It was also shown that LysE contributes to cadaverine export in *C. glutamicum* (Stäbler et al. 2011).

Putrescine PotE is a member of the APC family and mediates the uptake and excretion of putrescine in *E. coli*. Like CadB, the uptake of putrescine occurs by a proton-linked symport and the excretion is done in antiport mode with L-ornithine as further substrate (Kashiwagi et al. 1992; Kashiwagi et al. 1997). In addition, PuuP, a member of the same family, is known to be involved in the uptake of putrescine (Kurihara et al. 2009). The ABC transporter PotFGHI also catalyzes putrescine uptake (Pistocchi et al. 1993). Two putative putrescine importers, YdcSTUV and YeeF, are encoded in the genome of *E. coli* K12-MG1655, and recently, the uptake of putrescine by YeeF was shown (Kurihara et al. 2011). In *E. faecalis*, which is able to use agmatine as a sole carbon source, an agmatine-putrescine antiporter has been described (Driessen et al. 1988).

The putrescine precursors L-ornithine and L-arginine are transported in many ways across the cell membrane. The same proteins, which are responsible for L-lysine uptake, typically also use L-ornithine as a substrate (Fig. 3). The export of L-ornithine has been less investigated in *E. coli*, but a putative arginine-ornithine antiporter ArcD is encoded. In *E. coli*, ArgO, a member of the LysE superfamily, catalyzes efflux of L-arginine and possibly also of L-lysine and canavanine (Nandineni and Gowrishankar 2004). In *C. glutamicum*, LysE is required both for export of L-lysine and of L-arginine (Bellmann et al. 2001). Uptake of L-arginine may occur via the ABC transporters ArtPMQJI as well as ArgT-HisPMQJ (Wissenbach et al. 1995). A putative L-arginine uptake system is encoded by cg3045 in *C. glutamicum* (Ren and Paulsen 2005). AdiC constitutes an arginine-agmatine antiporter of the APC family, which plays a role in acid resistance of *E. coli* like PotE and CadB (Gong

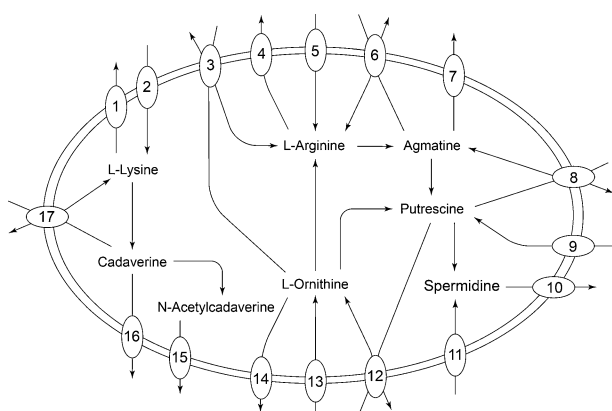


Fig. 3 Polyamine transport across the cell membrane in Bacteria. The known transport reactions mediating the uptake or secretion of polyamines, derivatives and precursors are shown. Genes encoding

No.	TC-number	<i>E. coli</i>	<i>C. glutamicum</i>	
		encoded by	TC-number	encoded by
1	2.A.76.1.3	yahN	2.A.75.1.1	lysE
2	2.A.3.1.2, 3.A.1.3.1	lysP, argT/hisJQMP	2.A.3.1.2	lysI
3	n.d.	arcD	n.d.	n.d.
4	2.A.75.1.2	argO	2.A.75.1.1	lysE
5	3.A.1.3.3, 3.A.1.3.1	artPIQM/artJ, argT/hisJQMP	n.d.	cg3045
6	2.A.3.2.5	adiC	n.d.	n.d.
7	n.d.	n.d.	?	?
8	n.d.	n.d.	n.d.	n.d.
9	2.A.3.1.13, 3.A.1.11.2, 2.A.3.-	puuP, potFGHI, yeeF	n.d.	n.d.
10	2.A.7.1.9	mdtJI	n.d.	n.d.
11	3.A.1.11.1	potABCD	n.d.	n.d.
12	2.A.3.2.1	potE	n.d.	n.d.
13	2.A.3.1.2, 3.A.1.3.1	lysP, argT/hisJQMP	n.d.	n.d.
14	?	?	?	?
15	n.d.	n.d.	?	?
16	n.d.	n.d.	2.A.75.1.1	lysE
17	2.A.3.2.2	cadB	?	?

corresponding transport-systems are given for *E. coli* and *C. glutamicum*. Question marks indicate obviously present, but uncharacterized transport processes; n.d. not determined

et al. 2003). Agmatine excretion was also observed in *C. glutamicum* when arginine decarboxylase genes were over-expressed (Schneider and Wendisch 2010), but the transport mechanism is still unclear in this bacterium.

Spermidine In *E. coli*, proteins responsible for uptake and excretion of spermidine are known. The uptake of spermidine is mediated by PotABCD (Furuchi et al. 1991) and the excretion by MdtJI (Higashi et al. 2008). PotABCD is a member of the ABC superfamily and catalyzes ATP-driven spermidine uptake. MdtJI belongs to the drug/metabolite transporter (DMT) superfamily, members of which are usually responsible for excretion of toxic compounds or waste metabolites.

Metabolic engineering for putrescine production

Putrescine can be overproduced from L-arginine and L-ornithine via the ADC and ODC pathway, respectively. However, the ODC pathway is preferable as it comprises only a single reaction compared to two or three reactions of the ADC pathway. To increase L-ornithine formation, its conversion to L-arginine may be blocked; however, this results in unfavorable auxotrophy for L-arginine. Thus, the maintenance of prototrophy with concomitant high L-ornithine supply is a focus in strain construction.

The pathway for biosynthesis of L-arginine and L-ornithine, the substrates of the initial decarboxylase reactions in the ADC and ODC pathway, respectively, are similar in *E. coli* and *C. glutamicum*. L-glutamic acid is converted in eight enzymatic steps to L-arginine. Five steps involving *N*-acetylated intermediates lead to L-ornithine, and three additional steps are required to convert L-ornithine to L-arginine. The differences between *E. coli* and *C. glutamicum* in this pathway are as follows. In the so-called “cyclic pathway” for ornithine synthesis in *C. glutamicum*, two steps are catalyzed by ornithine acetyltransferase (OAT, ArgJ, EC 2.3.1.35), as it converts L-glutamic acid and *N*-acetyl-L-ornithine to *N*-acetyl-L-glutamic acid and L-ornithine (Glansdorff and Xu 2007). In *E. coli*, the so-called “linear pathway” is found (Glansdorff and Xu 2007) with *N*-acetylglutamic acid synthase (ArgA, EC 2.3.1.1) and acetylornithinase (AO, ArgE, EC 3.5.1.16) replacing the two steps catabolized by ArgJ from *C. glutamicum*. As ArgJ recycles the acetyl group between ornithine and glutamic acid, it provides a more economical pathway in terms of metabolic costs for ornithine synthesis than the alternative route via ArgA and ArgE, which leads to concomitant hydrolysis of acetyl-CoA to acetic acid and CoA. The ornithine carbamoylphosphatetransferase ArgF (EC 2.1.3.3) converts L-ornithine to L-citrulline. The genome of *E. coli* K12-MG1655 contains two genes, *argI* (b4254)

and *argF* (b0273), both of which encode a functional carbamoylphosphatetransferase. ArgI and ArgF interact to form four trimeric isoenzymes (Legrain et al. 1972). The genome of *C. glutamicum* comprises only one carbamoylphosphatetransferase encoded by *argF* (cg1584). The first enzyme of the linear pathway, e.g., ArgA in *E. coli*, as well as *N*-acetylglutamic acid kinase (ArgB, EC 2.7.2.8) in *C. glutamicum* are subject to feedback regulation by L-arginine. Additionally, the synthesis of all enzymes in the pathway is subject to repression by L-arginine, which is mediated by the repressor ArgR in *E. coli* and *C. glutamicum* (Glansdorff and Xu 2007).

As mentioned above, a prerequisite for successful use of microorganisms in industrial fermentations or bio-transformations is a high tolerance of the desired product. Tolerance tests with *C. glutamicum* and *E. coli* showed very promising results. Concentrations of up to 66 g/l reduced the growth rate of *C. glutamicum* by 34% and that of *E. coli* by 78% (Schneider and Wendisch 2010). The biomass formation was reduced at the same concentration by 39% and 63%, respectively (Schneider and Wendisch 2010). If also effects of the counterion of putrescine (the dichloride salt of putrescine was used) have to be taken into account, tolerance of putrescine may even be higher.

Engineering of *E. coli* *E. coli* possesses two isozymes for both, ornithine decarboxylase (biosynthetic SpeC, degradative SpeF) and arginine decarboxylase (biosynthetic SpeA, degradative AdiA). The ADC pathway is completed by the agmatinase SpeB, which hydrolyzes agmatine to putrescine and urea. While urea cannot be reused by *E. coli*, putrescine can be utilized by *E. coli* as a sole carbon source on two pathways. The first pathway via γ -glutamylputrescine seems to be responsible for using putrescine as a carbon source, whereas the second pathway via γ -aminobutanol seems to be negligible (Kurihara et al. 2008).

The overexpression of *speC* (b2965) and of *speF* (b0693) in the wild-type genetic background led to comparable results as 0.72 or 0.87 g/l of putrescine accumulated in batch cultures (Eppelmann et al. 2006). The simultaneous overexpression of *speF* and *speAB* (b2938, b2937) slightly increased putrescine accumulation to 1.03 g/l. In fed-batch cultivation, the *E. coli* (*speF*) strain produced up to 5.1 g/l putrescine (Table 1) (Eppelmann et al. 2006).

The amount of secreted putrescine was further increased by engineering the genetic background of the host. The combined deletion of *argI* (Fig. 4, C-1; lowering the flux from L-ornithine towards L-arginine), *speE* (b0121, reducing putrescine consumption by spermidine synthase), *speG* (b1584, spermidine acetyltransferase which was active with putrescine at least in vitro), and *puuPA* (b1296, b1297,

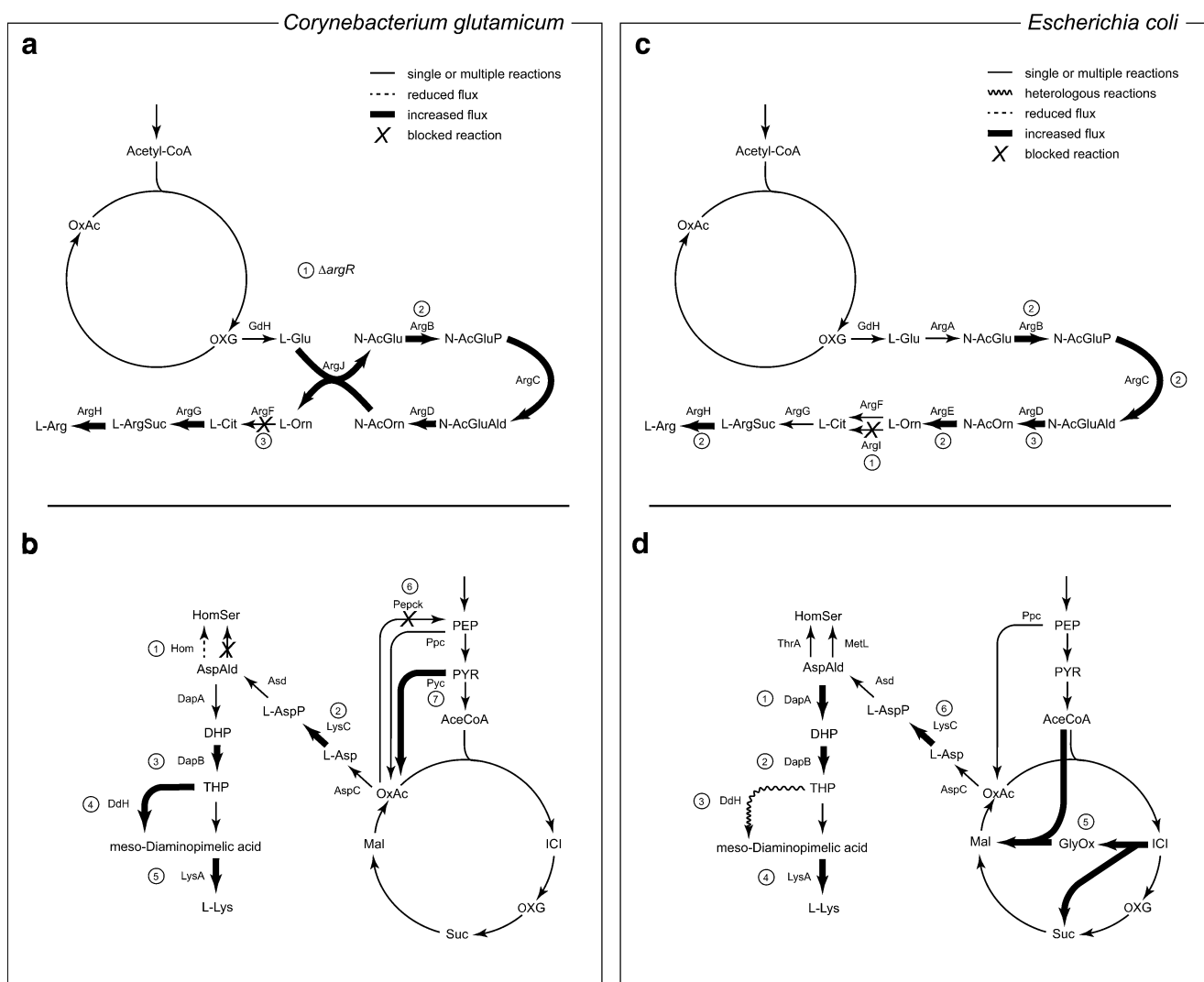


Fig. 4 Metabolic engineering targets in the central metabolism applied in **a, c** putrescine and **b, d** cadaverine producing strains of *C. glutamicum* and *E. coli*. Genetic modifications are numbered and the relevant explanations are given within the text. *OxG* 2-oxoglutaric acid, *L-Glu* L-glutamic acid, *N-AcGlu* N-acetylglutamic acid, *N-AcGluP* N-acetylglutamylphosphate, *N-AcGluAld* N-acetylglutamatic acid semialdehyde, *N-AcOrn* N-acetylornithine, *L-Orn* L-ornithine, *L-Cit* L-citulline, *L-ArgSuc* L-argininosuccinic acid, *L-Arg* L-arginine, *Suc* succinic acid, *Mal* malic acid, *OxAc* oxalic acid, *L-Asp* L-aspartic acid, *L-AspP* L-aspartylphosphate, *AspAld* aspartic acid semialdehyde, *HomSer* homoserine, *DHP* L-2,3-dihydropicolinic acid, *THP* tetrahydropicolinic acid, *L-Lys* L-lysine, *GlyOx*, *iciR* isocitrate lyase regulator, *argR* arginine repressor, *GdH* glutamic acid dehydrogenase (EC 1.4.1.4), *ArgA* amino acid N-acetyltransferase (EC 2.3.1.1), *ArgJ* bifunctional ornithine acetyltransferase/N-acetylglutamic acid synthase

putrescine importer and initial enzyme in putrescine degradation pathway via γ -glutamylputrescine) led to a putrescine titer of 1.18 g/l (Qian et al. 2009). A further optimization by 25% was achieved by promoter exchange of genes encoding the enzymes converting L-glutamic acid into L-ornithine (Fig. 4, C-2, C-3) as well as the exchange

(EC 2.3.1.35/2.3.1.1), *ArgB* acetylglutamic acid kinase (EC 2.7.2.8), *ArgC* N-acetylglutamylphosphat reductase (EC 1.2.1.38), *ArgD* acetylornithine aminotransferase (EC 2.6.1.11), *ArgE* acetylornithinase (EC 3.5.1.16), *ArgF* ornithine carbamoyltransferase (EC 2.1.3.3), *ArgG* argininosuccinic acid synthetase (EC 6.3.4.5), *ArgH* argininosuccinic acid lyase (EC 4.3.2.1), *Pepck* phosphoenolpyruvic acid carboxylase (EC 4.1.1.32), *Ppc* phosphoenolpyruvic acid carboxylase (EC 4.1.1.31), *Pyc* pyruvic acid carboxylase (EC 6.4.1.1), *AspC* aspartic acid aminotransferase (EC 2.6.1.1), *LysC* aspartokinase (EC 2.7.2.4), *Asd* aspartic acid semialdehyde dehydrogenase (EC 1.2.1.11), *MetL*, *ThrA* bifunctional aspartokinase/homoserine dehydrogenase (EC 2.7.2.4/1.1.1.3), *DapA* dihydrodipicolinic acid synthase (EC 4.2.1.52), *DapB* dihydrodipicolinic acid reductase (EC 1.3.1.26), *DdH* meso-diaminopimelic acid dehydrogenase (EC 1.4.1.16), *LysA* diaminopimelic acid decarboxylase (EC 4.1.1.20)

of *speF*–*potE* promoter (*potE* encodes the ornithine–putrescine antiporter) (Qian et al. 2009). The exchange of the *argA* promoter failed and deregulation of the transcriptional units *argECBH* and *argD* by deletion of *argR* even decreased production (Qian et al. 2009). The deletion of *rpoS* (b2741), encoding a global stress regulator, which was

chosen due to the assumption that putrescine overproduction might lead to a stress response, led to a 7% increase in product concentration (Qian et al. 2009).

The activity of ornithine decarboxylases is regulated on multiple levels. Besides feedback inhibition by L-ornithine, putrescine, and spermidine, the activity of ornithine decarboxylases is stimulated by GTP and inhibited by ppGpp, an alormone of the stringent response (Tabor and Tabor 1985). In *E. coli*, L-ornithine decarboxylase is also regulated by the antizyme AtoC (Filippou et al. 2007). Deletion of *atoC* (b2220) led to a slight increase in putrescine production, however, at the expense of the ability to grow to high cell densities (Qian et al. 2009), which might be due to the other physiological functions of AtoC.

Plasmid-based overexpression of the putrescine exporter gene *potE* failed so far, but the promoter exchange of the *potE-speF* operon successfully increased putrescine production, as mentioned above. As both *speF* and *potE* are affected, it is impossible to distinguish between positive effects due to increased levels of putrescine export or ornithine decarboxylase.

Engineering of *C. glutamicum* The metabolism of polyamines has not been investigated in *C. glutamicum*. In contrast to *E. coli*, *C. glutamicum* is unable to degrade and utilize putrescine as a carbon source (Schneider and Wendisch 2010). In order to overproduce putrescine, heterologous genes were expressed. The expression of genes of the ADC and ODC pathway from *E. coli* in the wild-type background of *C. glutamicum* only led to minor amounts of putrescine (Schneider and Wendisch 2010). As *C. glutamicum* is able to reuse urea, which is released by the agmatinase reaction, the feasibility of the ADC pathway was further investigated. Deletion of *argR* (Fig. 4, A-1) in combination with plasmid-based overproduction of feedback-insensitive ArgB (Fig. 4, A-2) led to substantial amounts of L-arginine. However, the combinatorial expression of genes for ADC enzymes clearly indicated that L-arginine decarboxylase and agmatinase limited the overall reaction. In the supernatant, 1.6 g/l L-arginine and 1.7 g/l agmatine, but only 0.1 g/l putrescine could be detected (Schneider and Wendisch 2010). No differences between the biosynthetic and degradative arginine decarboxylases were observed (Schneider and Wendisch 2010).

The deletion of *argR* and *argF* (Fig. 4, A-3) led to accumulation of L-ornithine but rendered the resulting strain arginine auxotrophic. When *speC* and *speF* from *E. coli* were expressed in the *argR-argF* deletion strain, production of 5 g/l putrescine resulted, which was about 50 times higher than strains endowed with the ADC pathway. As only minor amounts of L-ornithine were observed towards the end of the cultivation and as the in

vitro activity of SpeF was 24-fold higher than that of SpeC, it was tested whether supply of the decarboxylase cofactor pyridoxal phosphate (PLP) might be limiting. However, the addition of PLP to the medium did not increase putrescine production (Schneider and Wendisch 2010) indicating that L-ornithine supply might be limiting putrescine formation.

Metabolic engineering for cadaverine production

Cadaverine can be overproduced by introduction of an overproduced lysine decarboxylase. The corresponding substrate, L-lysine, is synthesized in *E. coli* and *C. glutamicum* by similar pathways covering ten enzymatic steps initiating from the TCA-cycle intermediate oxaloacetate. The three initial steps in this pathway lead to aspartic acid semialdehyde, which is the branch point for biosynthesis of the amino acids L-methionine, L-threonine, L-isoleucine, and L-lysine.

In *E. coli*, three aspartic acid kinases (EC 2.7.2.4) exist (LysC, ThrA, and MetL), which phosphorylate L-aspartic acid to L-aspartyl-phosphate. ThrA and MetL are bifunctional enzymes and also have homoserine dehydrogenase activity. By contrast, *C. glutamicum* possesses LysC as only aspartic acid kinase. The presence of a diaminopimelic acid dehydrogenase (EC 1.4.1.16, cg2900) in *C. glutamicum* is another difference. This enzyme catalyzes the direct conversion of tetrahydropicolinic acid to meso-diaminopimelic acid. Feedback inhibition by L-lysine is observed for LysC and dihydrodipicolinic acid synthase (DapA) (Park and Lee 2010). LysC from *C. glutamicum* is additionally feedback-inhibited by L-threonine, whereas ThrA from *E. coli* is subject to feedback-inhibition by L-threonine (Park and Lee 2010). In *E. coli*, *lysC* and *thrA* are controlled by transcriptional attenuation by L-threonine and L-lysine (Park and Lee 2010). The third aspartic acid kinase from *E. coli* encoded by *metL* is controlled by the MetJ repressor mediated by L-methionine (Park and Lee 2010).

The tolerance of *E. coli* for cadaverine seems to be lower compared to putrescine. The biomass formed in the presence of 51 g/l cadaverine was reduced by 30% in comparison to the same molar concentration of putrescine (Qian et al. 2011, Qian et al. 2009). This is an example of different polyamine toxicity, which might depend on the carbon chain length and the number of amino groups. *C. glutamicum* was only tested for growth on solid medium and grew even at concentrations of up to 31 g/l (Mimitsuka et al. 2007).

Engineering *E. coli* The first report on cadaverine production by use of microorganisms was published in 2007 (Nishi et al. 2007). The inventors overexpressed the lysine decarboxylase gene *cadA* (b4131) in *E. coli* and used resting cells to produce 69 g/l cadaverine (Table 1).

The plasmid-based overexpression of *cadA* in the wild-type genetic background led to accumulation of 0.8 g/l cadaverine by growing cells (Qian et al. 2011). Interestingly, the authors reported that repeated attempts to overexpress the biosynthetic lysine decarboxylase *ldcC* (b0186) always failed. The cloning of *cadA* instead led in repeated trials to mutated versions of *cadA* with a 5' insertion (Qian et al. 2011). The author suggested that this insertion might form a stable stem loop in front of the start codon, which sequesters the ribosome binding site and, thus, lowers the translation efficiency.

To avoid side reactions of enzymes active with putrescine towards cadaverine, a number of genes were deleted: the spermidine synthase gene *speE*, the spermidine acetyltransferase gene *speG*, the putrescine importer gene *puuP*, the putrescine amino transferase gene *puuA* and *ygjG*, which encodes the initial enzyme of the second putrescine degradation pathway and is known to be active in vitro with cadaverine (Qian et al. 2011). The resulting strain was able to accumulate 1.2 g/l cadaverine. Production of cadaverine was increased by 10% as a consequence of enhancing the flux of L-aspartic acid towards L-lysine by overexpression of *dapA* via promoter exchange (Fig. 4, D-1). In fed-batch cultivation, this strain produced 9.6 g/l cadaverine (Qian et al. 2011). Overexpression of *lysC* (Fig. 4, D-6) or use of a feedback insensitive LysC variant did not increase productivity (Qian et al. 2011). Similarly, combined overexpression of *dapB* (b0031, Fig. 4, D-2) and *lysA* (b2838, Fig. 4, D-4), the increased activity of the glyoxylate shunt (Fig. 4, D-5), and the heterologous expression of *ddh* (cg2900, Fig. 4, D-3, 5) from *C. glutamicum* did not further improve cadaverine formation. Therefore, the authors assumed that precursor supply, oxaloacetic acid from the TCA cycle, might be limiting (Qian et al. 2011).

Engineering *C. glutamicum* Heterologous expression of *cadA* from *E. coli*, and inactivation of the homoserine dehydrogenase gene *hom* (cg1337, Fig. 4, B-1) was achieved by insertion of *cadA* into *hom*. The expression of *cadA* was driven by the strong kanamycin resistance gene promoter. The resulting strain accumulated 2.6 g/l cadaverine in the supernatant, but was auxotrophic for L-methionine, L-threonine, and L-isoleucine (Mimitsuka et al. 2007). Moreover, this strain still accumulated substantial amounts of L-lysine, which could be overcome by plasmid-based expression of *cadA*. In a different approach, the authors opted for the biosynthetic lysine decarboxylase LdcC from *E. coli* because of its near neutral pH optimum. Overexpression of *ldcC* led to 30% more cadaverine than overexpression of *cadA* (Kind et al. 2010b). The flux towards L-lysine was improved by amplification of a gene for feedback-insensitive aspartokinase (*lysC*) and overexpression of *dapB* (dihydrodipicolinate reductase), *ddh* (diaminopimelate dehydroge-

nase), and *lysA* (diaminopimelate decarboxylase), drain to side-products was reduced by introducing *hom* leaky alleles (Fig. 4, B-2,3,4,5), and oxaloacetate supply was strengthened by expression of a gene encoding a variant of pyruvate carboxylase (*pyc*^{P458S}), the major anaplerotic enzyme in *C. glutamicum* (Peters-Wendisch et al. 2001; Peters-Wendisch et al. 1998) as well as by deletion of the gene for phosphoenolpyruvate carboxykinase (Fig. 3, B-6,7) (Riedel et al. 2001). By integration of codon optimized *ldcC* into the genome of this strain, an increase of 20% was achieved (Kind et al. 2010b). This was further increased by 35% due to addition of the lysine decarboxylase cofactor PLP, indicating this as one bottleneck in cadaverine production by this strain (Kind et al. 2010b).

N-acetylcadaverine was formed as a byproduct of cadaverine production (up to 20%). Among 17 possible *N*-acetyltransferases encoded in the genome of *C. glutamicum*, the enzyme encoded by *cg1722* was found to be responsible for this reaction as deletion of *cg1722* led to a yield increase of 11% and *N*-acetylcadaverine could no longer be detected in the supernatant (Kind et al. 2010a).

Conclusion and perspective

In this mini-review, we outlined the anabolic and catabolic polyamine pathways known so far in *Bacteria* and *Archaea* as well as the transport processes of polyamines, their precursors and derivatives across the cell membrane. This knowledge has recently been applied to engineer *C. glutamicum* and *E. coli* strains for cadaverine and putrescine production as described above. But where do we go from here?

The production of putrescine and cadaverine benefits both from single thermodynamically favorable decarboxylation reactions and from the wealth of knowledge about precursor amino acid biosynthesis in these organisms. Together, this led to reasonable titers and productivities (Table 1). However, the effect of several known engineering targets has not been investigated in putrescine and cadaverine production strains until now. For example, the engineering for putrescine production focused on reactions downstream of L-glutamic acid. The effect of deregulation of the 2-oxoglutaric acid dehydrogenase complex (ODHC), which has been shown to be relevant for L-glutamic acid overproduction (Shimizu and Hirasawa 2007), remains uninvestigated. For L-lysine production, the effect of NADPH supply is known to be important (Wittmann and Becker 2007), which remains also uninvestigated in cadaverine production strains. Hence, there are still engineering targets known which might be worth investigating in putrescine and cadaverine overproducing strains.

Higher polyamines such as the triamines aminopropylcadaverine and spermidine are derived directly from cadaverine and putrescine, respectively (Fig. 2; reactions 11, 22). However, all known aminopropyltransferases require decarboxylated *S*-adenosylmethionine (dcSAM) as the aminopropyl donor. In these organisms, dcSAM is generated from *S*-adenosylmethionine (SAM), a central intermediate of sulfur and C1 metabolism involved in many crucial reactions. Its synthesis is costly as three ATP equivalents are required for transferring adenine from ATP to *L*-methionine. Moreover, 5'-methylthioadenosine (MTA) formed as co-product of dcSAM-dependent aminopropyltransferase reactions has to be recycled and the only known pathway for MTA recycling involves a multiple reaction cascade leading to adenine and *L*-methionine (Sekowska and Danchin 2002). Because of the energy requirements, the complex cofactor recycling pathway, and the intricate network of SAM converting pathways, metabolic engineering for the overproduction of polyamines involving SAM-dependent aminopropyltransferases is extremely challenging.

The approaches described here focused exclusively on bacterial pathways. However, the potential of archaeal and eukaryotic pathways to polyamines may be tapped in the future within the framework of synthetic biology.

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