Review

Nitrogen Degradation Pathways in Actinomycetes: Key Components of Primary Metabolism Ensuring Survival in the Environment

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Nitrogen Degradation Pathways in Actinomycetes: Key Components of Primary Metabolism Ensuring Survival in the Environment

Sergii Krysenko

Valent BioSciences, Biorational Research Center, 1910 Innovation Way, Suite 100, Libertyville, Illinois 60048, USA; sergii.krysenko@valentbiosciences.com; Tel.: +1 (224) 563-7863

Abstract

Nitrogen is an essential element required for bacterial homeostsis. It serves as a building block for the biosynthesis of macromolecules and provides precursors for secondary metabolites. Actinomycetes have developed the ability to use various nitrogen sources and possess two central enzyme systems for nitrogen assimilation involving glutamine synthetase / glutamate synthase and glutamate dehydrogenase. Microorganisms living in habitats with changeable availability of nutrients have to cope with limited nitrogen availability in their ecological niches and developed strategies to survive under nitrogen limitation. A complex nitrogen metabolism of Actinobacteria allows the utilization of various compounds as N-sources. One such adaptation is the ability to acquire nitrogen from alternative sources like monoamines or polyamines putrescine, cadaverine, spermidine and spermine, ensuring both nutrients availability (C- and N-source) and resistance against high polyamine concentrations. Bacterial polyamine catabolism is not only important under low nitrogen availability, but it is also required to survive under high concentrations of these compounds. Such conditions can occur in diverse habitats like soil, plant tissues and human cells. Strategies of pathogenic and non-pathogenic Actinobacteria to survive in the presence of mono- and polyamines offer the possibility to combat pathogens by using their capability to metabolize polyamines as an antibiotic drug target. This work aims to summarize the knowledge on nitrogen utilization and, more specifically, catabolism of amines in actinobacterial survival and its role in nitrogen metabolism.

Keywords: nitrogen metabolism; polyamine utilization; polyamine metabolism; bacterial survival; bacterial physiology; Actinobacteria

1. Nitrogen Metabolism in Prokaryotes

In addition to carbon, oxygen, and hydrogen, the main component of all living cells is nitrogen (N). It makes up about 14% of the cell's dry weight (Fuchs, 2022). It is an essential component for bacterial metabolism needed for the synthesis of purines and pyrimidines that are the basic building blocks of DNA and RNA, of amino acids that are required for protein synthesis, and of amino sugars that are components of cell walls. The basic building blocks often serve as precursor molecules for the formation of secondary metabolites (Aharonowitz, 1980; Reitzer, 2001; (Ninfa et al., 2001), which may contain atoms of nitrogen. For example, *Streptomyces* produced molecules of the antibiotic undecylprodigiosin (Red) contain three nitrogen atoms, while the calcium-dependent antibiotic contains 14 N atoms (Hojati et al. 2002; Hopwood, 1988). The availability of nitrogen plays a crucial role in primary metabolism and in secondary metabolite formation (Krysenko & Wohlleben, 2025).

Bacteria from the phylum Actinobacteria can obtain the nitrogen they need for cellular metabolism from various sources. While soil bacteria can utilize both animal and plant remain, most enterobacteria depend on metabolites of animal metabolism. These include organic substances such as amino acids, creatinine, urea, or simple inorganic substances such as ammonium or nitrate

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(Neidhardt and Reitzer, 1996). The preferred nitrogen source for optimal growth in most bacteria is ammonium. For the uptake and utilization of nitrogen, bacteria have developed specific transporters along with mechanisms of assimilation and regulation. This allows them to respond quickly to changes in the environment and rapidly optimize their metabolism (Jacoby et al., 2020).

1.1. Nitrogen Uptake and Nitrogen Assimilation in Actinobacteria

Actinomycetes can utilize a wide range of substances as nitrogen sources. These include inorganic compounds such as ammonium, nitrate, and nitrite, as well as organic compounds, such as amino acids (e.g. histidine and arginine) and amino sugars (Magasanik, 1982; Hopwood, 1999). Nitrogen uptake in Actinobacteria has been extensively investigated in the model bacterium *Streptomyces coelicolor*. *S. coelicolor* lives in the soil under variable N- and C-conditions and therefore possesses the ability to metabolize a variety of different C- and N-sources, including amino sugars, amino acids, amines (mono- and polyamines), peptides, urea, NO3-, NH4. Ammonium is the preferred nitrogen source and leads to higher bacterial growth rates overall than other nitrogen sources (Merrick & Edwards, 1995). This is because ammonium can be directly absorbed into the cellular cycle.

All other inorganic compounds coming into the cell must first be reduced to ammonium at the expense of energy. Complex organic substances must be broken down by intracellular and/or extracellular enzymes. Various amino acids can be made available by deamination, which produces free ammonium, or transamination, i.e., the transfer of the amino group to, for example, 2-oxoglutarate (Voelker & Altaba, 2001). Depending on the available nutrient, bacteria can specifically synthesize or activate the necessary proteins for transport and degradation of the substances (Merrick & Edwards, 1995). Glutamine synthetases (GSs) play a key role in cellular nitrogen metabolism. Due to their high substrate specificity, these enzymes are capable of assimilating ammonium at concentrations below 0.1 mM (Magasanik, 1982).

Glutamine is formed from ammonium and glutamate using ATP. Glutamate synthase (GOGAT) then catalyzes the NADPH-dependent formation of glutamate from glutamine and 2-oxoglutarate. The GS/GOGAT pathway is ubiquitous in bacteria and is the only pathway for ammonium utilization in many organisms. Glutamine serves as both an amino acid and a nitrogen donor in the synthesis of approximately 25% of all nitrogen-containing cellular components (Reitzer & Schneider, 2001). Some bacteria have an alternative route for ammonium assimilation: the direct formation of glutamate through reductive amination of 2-oxoglutarate by glutamate dehydrogenase (GDH). However, due to its low substrate specificity, this enzyme only works effectively under high ammonium concentrations (Figure 1) (Merrick & Edwards, 1995).

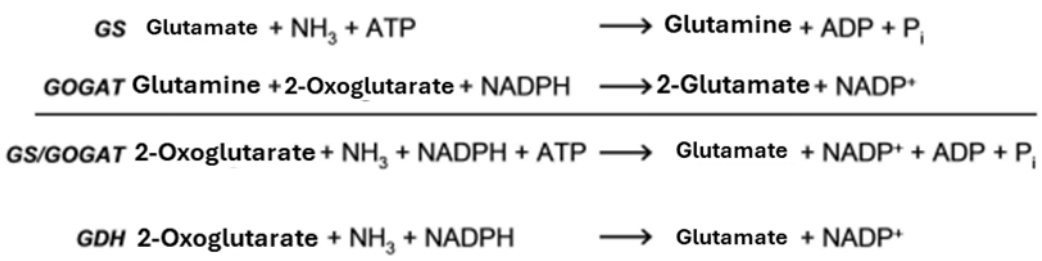

Figure 1. Central reactions of ammonium assimilation. At low concentrations, ammonium can be introduced into metabolism via the GS/GOGAT pathway. In the net reaction, glutamate is formed from 2-oxoglutarate and ammonium, consuming ATP and NADPH. At high concentrations, GDH catalyzes ammonium assimilation. All nitrogen-containing cellular components and metabolites, such as purines, pyrimidines, amino sugars, amino acids, and proteins, are produced from glutamine and glutamate (Zalkin and Smith, 1998). GS: glutamine synthetase, GOGAT: glutamate synthase, GDH: glutamate dehydrogenase.

1.1.2. Nitrogen Assimilation in Actinomycetes: ammonium catabolism as central catabolic route

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The regulation of N assimilation in *Actinomycetales* differs from that in *Enterobacteriaceae*, but also has numerous similarities, particularly in the key enzymes. It can occur at the transcriptional and post-translational levels. Proteins such as GlnR, GlnRII, AmtR, NnaR, and Crp play an important role in transcriptional regulation. Post-translational regulation occurs through the signaling protein PII and by modulating the activity of important enzymes (GDH, GS) through adenylation/uridylation. GSI and GOGAT in Actinomycetales fulfill the same function in ammonium assimilation as GS and GOGAT in *E. coli*: In their overall reaction, ammonium and α-ketoglutarate are converted to glutamate with the consumption of ATP and NADPH (Figure 2) (Wray and Fisher, 1988, 1991, 1993; Fisher, 1989).

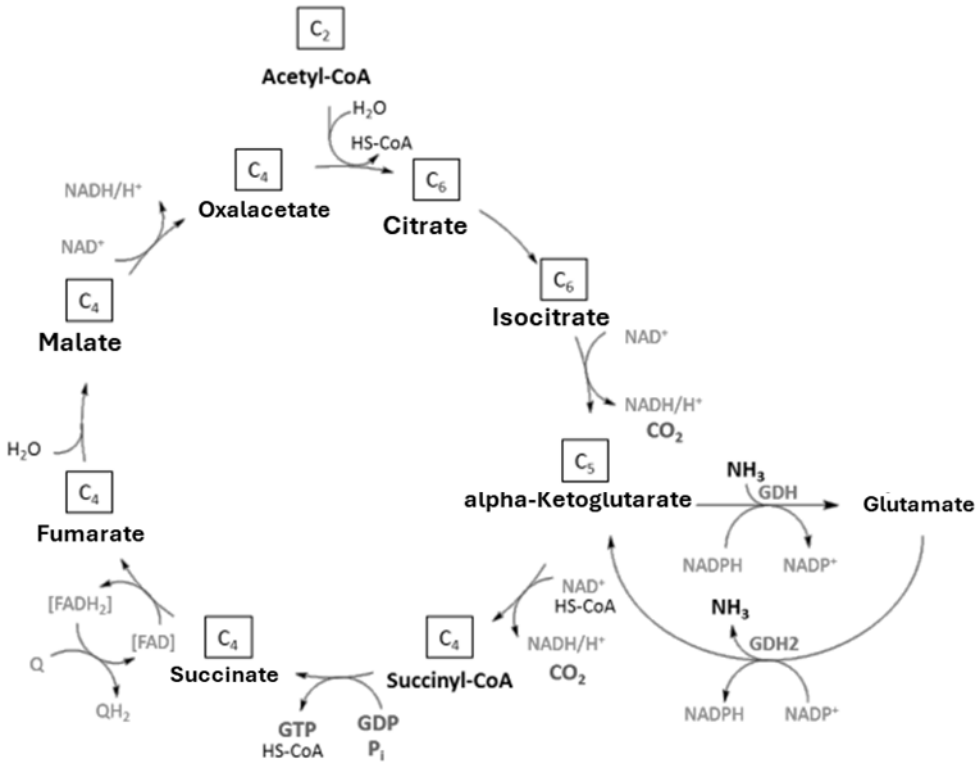

Figure 2. Krebs cycle or tricarboxylic acid (TCA) cycle with reactions of the anabolic and catabolic glutamate dehydrogenases (modified after Voelker & Altaba, 2001; Merrick & Edwards, 1995).

GSI is regulated by GlnE via adenylation and deadenylation (Streicher and Tyler, 1981; Fink et al., 1999; Nolden et al., 2001; Williams et al., 2013). Interestingly, there is evidence of possible transcriptional regulation by GlnE, which would make GlnE a so-called "moonlighting" protein (Rehm et al., 2010). Another form of glutamine synthetase, GSII (encoded by *glnII*), was first discovered in *Rhizobium* (Darrow and Knotts, 1977). GSII is a heat-labile octamer and shows sequence similarity to a eukaryotic GS. According to recent studies, GSII was introduced into the photosynthetic eukaryote Chloroplastida by horizontal gene transfer from gamma-proteobacteria at an early stage of plant evolution (Ghoshroy et al., 2010). GSII has been found in many members of the Actinomycetales (Edmands et al., 1987; Behrmann et al., 1990; Kumada et al., 1990), but it is absent in *Amycolatopsis*, *Mycobacterium*, and *Corynebacterium* (Reuther and Wohlleben, 2007). The difference from GSI is the lack of posttranslational regulation by adenylation (Hillemann et al., 1993). Instead, the GSII protein can degrade rapidly after N shock, suggesting control by proteolysis.

In addition to glutamine synthetases, bacteria possess GS-like proteins. GlnA2, GlnA3, and GlnA4 have been found in some Actinomycetes, e.g., in *S. coelicolor* (Bentley et al., 2002) and in *M. tuberculosis* (Harth et al., 2005). However, these proteins lack the amino acid residues conserved in GS, which form an adenylation motif. Therefore, a deficiency in the posttranslational regulation of

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GlnA2, GlnA3, and GlnA4 by adenylation was suspected. The transcription of *glnA2*, *glnA3*, and *glnA4* is generally not regulated by GlnR or GlnRII in *S. coelicolor*, as is the case with *glnA* and *glnII* (Fink et al., 2002; Rexer et al., 2006). These proteins also lack the amino acid residues involved in the catalytic reaction of GS. Accordingly, GlnA2, GlnA3, and GlnA4 did not exhibit GS activity (Krysenko et al., 2017). The function of the GS-like protein AtdA1 in *Acinetobacter sp.* strain YAA lies in the ATP-dependent reaction of glutamate and aniline to ɤ-glutamylanilide, which serves for aniline degradation (Takeo et al., 2013).

On the other hand, transcription of *glnA2* is upregulated in the *glnR* mutant of M. smegmatis (Jenkins et al., 2013; Jessberger et al., 2013). Not all bacteria function in parallel with GSI. For example, in *B. subtilis*, only the GS pathway is used (Deshpande et al., 1981). In contrast, in *S. hygroscopicus* 155, another enzyme, alanine dehydrogenase (ADH), is used instead of GDH for ammonium assimilation (Chipeva et al., 1991). ADH catalyzes the amination of pyruvate, consuming NAD+ and leading to alanine formation. Because ADH has a high Km value, it is particularly beneficial to bacteria at high N concentrations, such as 20–100 mM ammonium (Chipeva et al., 1991). ADH activity has also been detected in *S. clavuligerus* (Aharonowitz, 1980; Bascaran et al., 1986) and in *R. leguminosarum* (Allaway et al., 2000). In some *Streptomyces*, the activity of both enzymes, ADH and GDH, has been demonstrated (Shapiro and Vining, 1983).

In contrast to *E. coli*, which possesses only the NADPH-dependent GDH (Tyler, 1978), some Actinomycetes contain two GDHs. Glutamate dehydrogenase catalyzes the conversion of ammonium and α-ketoglutarate to glutamate (reductive amination) as well as the reverse reaction. In prokaryotes, ammonium assimilation is usually catalyzed by an anabolic, NADPH-dependent GDH enzyme (Hudson and Daniel, 1993). Glutamate cleavage (oxidative deamination) during ammonium dissimiliation is carried out by a catabolic, NADH-dependent glutamate dehydrogenase. The specificity of the enzymes toward their coenzymes NADP+ or NAD+ has been linked to conserved amino acid residues: an acidic amino acid at position 7 (P7) acts as an indicator of NADP+ specificity (Engel, 2014). The anabolic GDH is a homohexamer with approximately 50 kDa subunits. The catabolic prokaryotic GDH2 was discovered later than the anabolic GDH. A characteristic feature of GDH2 is its large subunit of 180 kDa (Kawakami et al., 2007).

1.1.2.1. Nitrogen Assimilation in Actinomycetales: catabolism of poor nitrogen sources

The assimilation of poor nitrogen sources requires the use of specialized enzymes involved in sophisticated systems for nitrate assimilation and respiration, for the assimilation of urea, amino acids, and other nitrogen-containing substances (Figure 3) (Hopwood et al., 1995, 1999). Nitrate can be either assimilated (extracted nitrogen is incorporated into cellular components in anabolic reactions) or dissimilated (in so-called nitrate respiration, nitrate is used as an electron acceptor instead of oxygen). Some Actinobacteria can operate both nitrate reduction pathways (Fischer et al., 2010, 2013). Nitrate uptake and utilization require special enzymes. First, nitrate molecules are transported into the cell by nitrite/nitrate transporters (NarK). There are two types of NarK transporters, NarK1 and NarK2. These are encountered as transport proteins in dissimilatory and assimilatory processes. The first is a nitrate/proton symporter, the second a nitrate/nitrite antiporter. Many proteobacteria use a mixed transporter in which NarK1 and NarK2 are fused together (Goddard et al., 2008, 2017).

After transporting into the cell, nitrate is reduced to nitrite. This is performed by a nitrate reductase. There are two classes of prokaryotic nitrate reductases: the Nar and Nas/Nap clades (Stolz and Basu, 2002). Cytosolic Nas reductase is involved in the assimilation of nitrate. In contrast, membrane-bound Nar and Nas reductases are involved in dissimilatory processes. The reducing equivalents for Nas can be derived from NAD(P)H, ferredoxin, or flavodoxin.

Transcription is induced by ammonium deficiency and nitrate presence (Stewart, 1994). Nas has the least conserved amino acid sequence among nitrate reductases, which is why it is considered a rapidly evolving protein (Stolz and Basu, 2002). In cyanobacteria, NarB functions as a cytoplasmic, assimilatory nitrate reductase. NarB, which belongs to the class of Nas reductases, has also been

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found in Actinomycetes. For example, NasA and NarB are present in some *Streptomyces* (Hsiao and Kirby, 2008; Fischer et al., 2014).

Nas, a related protein, is more flexible in its function. In most cases, it catalyzes the first step in the reduction of nitrate to ammonium. Furthermore, it can also play a role in denitrification reactions, the maintenance of redox balance, and nitrate sensing and uptake under severe N deficiency (Richardson et al., 2001). Nar is involved in the establishment of the proton gradient during nitrate respiration (Zumft, 1997).

Nitrite reductases convert nitrite produced in the reaction catalyzed by nitrate reductases into ammonium (assimilatory pathway) or into NO (dissimilatory pathway). Four types of nitrite reductases have been identified: Cu-containing NirK, found exclusively in denitrifying bacteria (Zumft, 1997; Higgins et al., 2016) and three other reductases with different heme groups (cytochrome cd1, siroheme, or multiheme) (Richter et al., 2002; Wang et al., 2000). NirS with the heme group cytochrome cd1 has been found only in denitrifying bacteria, while two nitrite reductases, NirB with siroheme and NrfA with multiheme, are present in *E. coli* (Wang et al., 2000; Besson et al., 2022)

In *S. coelicolor*, NnaR together with the global regulator GlnR, regulates the gene expression of *narK*, *nasA*, and *nirB* (Tiffert et al., 2011; Amin et al., 2012). The high sequence homology of NnaR in different Actinomycetales and the genetic location of nnaR, which was always found near *narK*, *nasA*, and *nirB*, suggest that NnaR plays a role in nitrate assimilation in all Actinomycetales.

In Actinomycetales, the genes for the ammonium transporter (encoded by *amtB*), the signaling protein PII (unlike in *E. coli*, PII is encoded by *glnK* instead of *glnB*), and the uridylyltransferase (encoded by *glnD*) are combined in an *amtB* operon (Wang et al., 2000; Jakoby et al., 2000; Fink et al., 2002). While the operon is inactive in *E. coli* under N excess (van Heeswijk et al., 1996), weakened expression of the amtB operon has been reported in *Streptomyces* under these conditions (Figure 3) (Tiffert et al., 2008).

In *Streptomyces* and *Mycobacteria*, GlnK is adenylated by GlnD under N deficiency and deadenylated again under N excess (Hesketh et al., 2002; Williams et al., 2013; Ensinck et al., 2024). In *E. coli*, GlnK is uridinylated and thus transmits the cellular N concentration to the ATase that regulates GSI activity (Atkinson and Ninfa, 1993, 1994, 1998, 2002; Jiang et al., 1998; Atkinson and Ninfa, 1999; Fink et al., 1999; Jiang and Ninfa, 2009; Radchenko et al., 2010, 2013). In Actinomycetales, GlnK is modified by the glutamine synthetase adenylyltransferase GlnE (ATase equivalent in Actinomycetales) (Fink et al., 1999; Forchhammer, 2007). However, signaling from GlnK to GlnE is absent in Actinomycetales (Williams et al., 2013), and the significance of GlnK adenylation is currently unknown. In addition, GlnK is *Streptomyces* by cleavage of the first three amino acids at the N-terminus after ammonium shock (Hesketh et al., 2002).

The functional versatility of GlnK in Actinomycetes is also evident in N metabolism systems such as the AmtR regulon of *Corynebacterium* (Jakoby et al., 2000) or the TnrA regulon of *Bacillus* (Fisher and Sonenshein, 1991). The AmtR repressor in *Corynebacteria* is not regulated by small effector molecules, as is common in the TetR family (Ramos et al., 2005). Instead, the repressor dissociates from the DNA after forming a complex with GlnK adenylated at Tyr51 (Jakoby et al., 2000; Nolden et al., 2001; Strösser et al., 2004; Beckers et al., 2005). Interestingly, AmtB plays a role in this process (Strösser et al., 2004). TnrA, the transcriptional regulator of N metabolism in *Bacillus*, is bound to a membrane-bound GlnK-AmtB complex in the absence of ATP (Heinrich et al., 2006).

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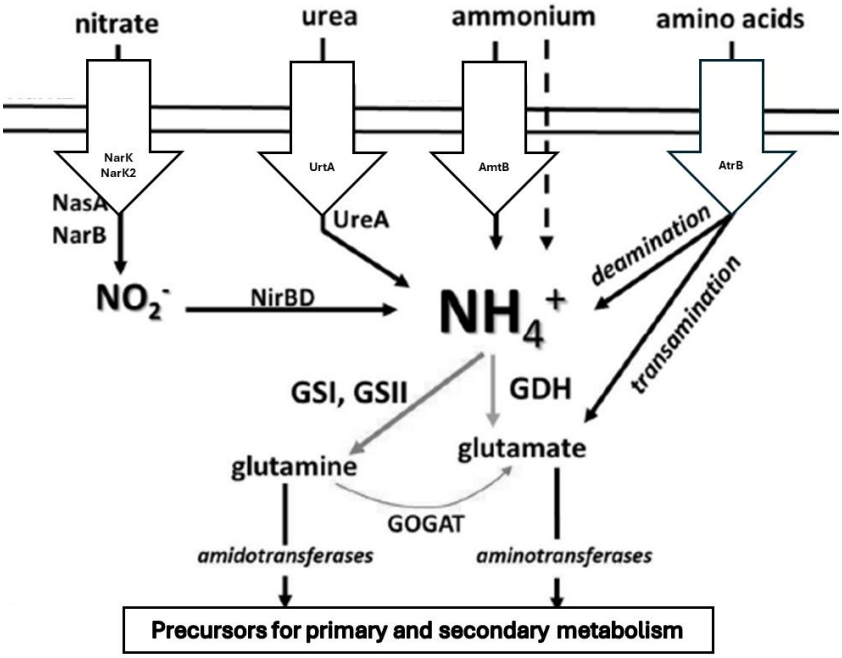

Figure 3. Pathways of ammonium, nitrate, urea and amino acids assimilation in *Streptomyces*. The uptake of various nitrogen sources into the metabolic cycle occurs via conversion to ammonium and its assimilation via the central GS/GOGAT or GDH pathways. GS: Glutamine synthetase, GOGAT: Glutamate synthase, GDH: Glutamate dehydrogenase (modified after Krysenko & Wohlleben, 2022).

1.1.2.2. Assimilation of Amines in Actinomycetes

Amines are organic compounds that contain carbon-nitrogen bonds, whereas one or more hydrogen atoms in ammonia are replaced by alkyl or aryl groups. The functional group −NH2 present in primary amines is called the amino group, which is present in biologically occurring amines, such as monoamines (e.g. ethanolamine) and polyamines (e.g. putrescine). Polyamines are cationic charged molecules with a hydrocarbon chain and multiple amino groups (Michael, 2018). They fulfill a lot of physiological functions, e.g. cell growth, maturation and proliferation, cell signaling, gene expression and others (Cohen, 1998; Igarashi & Kashiwagi, 2000; Kusano & Suzuki, 2015; Miller-Fleming et al., 2005). The monoamine ethanolamine is a short molecule which is both a primary amine and a primary alcohol and is a nitrogenous base in phospholipids and a building block of biomembranes (e.g. phosphoethanolamine) (Knaak et al., 1997). Excess of polyamines or ethanolamine is very toxic for bacterial cells and can lead to cell death (Miller-Fleming et al., 2015). Hence, polyamine and ethanolamine utilization represent bacterial survival strategies that allow survival of *S. coelicolor* in soil environment. Modified (e.g. glutamylated or acetylated) mono-/polyamines can be used in metabolism as sources of carbon and nitrogen.

In *S. coelicolor* putrescine, spermidine and diaminopropan biosynthesis have been described in the late-stationary phase in NMMP-medium, but cadaverine synthesis occurred only under iron limitation (Burell et al., 2012). Proteins with high similarity to the polyamine binding lipoprotein (PotD) and/or putrescine-binding periplasmic protein (PotF), to the amino acids/polyamine permease (PuuP) and/or putrescine importer (PlaP) were found in many phyla including Actinobacteria. SCO5667 is a predicted homolog of the putrescine-binding periplasmic protein (PotF) from *E. coli* with the sequence identity/similarity of 29/47%. However, transcriptional analysis of expression patterns of these genes revealed only weak expression of *sco5667* and *sco5671* in presence of spermidine (Krysenko et al., 2017, 2021).

The most likely steps of the polyamine utilization pathway in *S. coelicolor* were reported to be similar to that known in *E. coli* (Kurihara et al., 2005, 2008, 2013) and *P. aeruginosa* (Kwon et al., 2006).

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The initial step of the polyamine utilization is catalyzed by gamma-glutamylpolyamine synthetases GlnA2 and/or GlnA3 resulting in glutamylated polyamines (Krysenko et al., 2017, 2022). These glutamylated products can likely be further reduced by the predicted gamma-glutamylpolyamine oxidoreductase (SCO5671). The reaction results in the production of the gamma-glutamyl-gamma-aminopentanal or gamma-glutamyl-gamma-aminobutyraldehyde. The SCO5671 enzyme is a close ortholog of the gamma-glutamylpolyamine oxidoreductases PuuB from *E. coli* and PauB1-B4 from *P. aeruginosa*. The gene *sco5671* showed expression in presence of spermidine, but no expression in the presence of polyamines putrescine, cadaverine. In the next step of the utilization pathway, the predicted dehydrogenases (SCO5666 and SCO5657) might be involved. These proteins are predicted homologs of PuuC and PatD from *E. coli*, which are (gamma-glutamyl-) gamma-aminobutyraldehyde dehydrogenases. This step of the pathway may result in the production of the gamma-glutamyl-aminovalerate or gamma-glutamyl-GABA. The next step of polyamine utilization might require a predicted hydrolase (SCO6961) and result in the production of aminovalerate or GABA. Enhanced expression of *sco5666*, *sco5657* and *sco6961* in presence of polyamines was observed (Krysenko et al., 2017, 2021). These results suggest the role of SCO5666, SCO5657 and SCO6961 in polyamine utilization. Afterwards, SCO5676, which is a predicted homolog of the GABA aminotransferase GabT from *E. coli*, may be involved and catalyze the production of glutarate semialdehyde or succinate semialdehyde (Krysenko et al., 2021). It has been shown that the expression of the *sco5676* gene is strong in presence of arginine (Perez-Redondo et al., 2012). Arginine is a precursor of putrescine in *S. coelicolor* M145. This finding as well as enhanced expression of *sco5676* in presence of polyamines observed suggest the possible role of SCO5676 in the polyamine utilization. In the last step of the polyamine utilization pathway, SCO5679, which is a predicted homolog of the succinic semialdehyde dehydrogenase GabD from *E. coli*, may be involved. The polyamine utilization pathway ends with the succinate or glutarate that feeds the tricarboxylic acid (TCA) cycle (Figures 2 and 4) (Krysenko et al., 2021).

A predicted amidotransferase (SCO5655) was identified, which is a homolog of the putrescine amidotransferase (PatA) from *E. coli*. In transcriptional analysis, the expression of *sco5655*, *sco6960*, *sco6961* was enhanced in presence of polyamines. Moreover, *sco5655* was reported to be induced by a diamide (Kallifidas et al., 2010) and not by arginine (Perez-Redondo et al., 2012). No orthologs of SCO6961 and SCO6960 were found in *E. coli* or *P. aeruginosa*. These findings suggest the possibility of an alternative polyamine utilization pathway in *S. coelicolor* (Figure 4) (Krysenko et al., 2017).

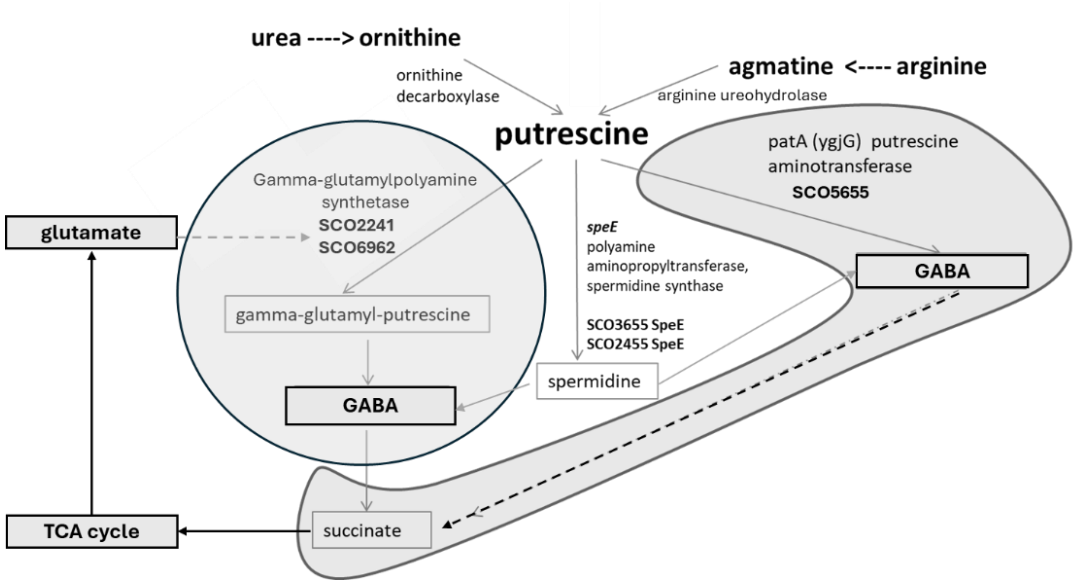

Figure 4. Model of the polyamine putrescine utilization in Actinomycetes with links to polyamine biosynthesis and TCA cycle - the case study model system *S. coelicolor* (modified after Krysenko & Wohlleben, 2022).

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Metabolism of monoamine ethanolamine has been investigated in such Actinobacteria as *Streptomyces* and *Mycobacterium* (Krysenko & Wohlleben, 2022). In *S. coelicolor*, predicted ethanolamine permeases (SCO6014 and SCO5977) possess likely other functions, because the expression of the genes *sco6014* and *sco5977* was not induced by ethanolamine (Krysenko et al., 2019). Likely, an ethanolamine permease encoding gene was not required and lost in *S. coelicolor* during the evolution process and enough ethanolamine may enter the cells very probably through diffusion. It is also possible that *S. coelicolor* uses ethanolamine utilization pathway to control intracellular ethanolamine level during decomposition of membranes and to recycle the N-source.

In order to utilize ethanolamine as an N- and C-source and balance the intracellular ethanolamine pool, bacteria had to develop metabolic pathways for ethanolamine utilization. The canonical ethanolamine utilization pathway was studied in *S. typhimurium* and *E. coli*. However, alternative ethanolamine utilization pathways were reported in *M. tuberculosis* and *C. salexigens*. These pathways do not require a metabolosome and do not result in the production of toxic intermediates, such as acetaldehyde (Krysenko & Wohlleben, 2022).

It was shown in a recent study that diverse organisms ranging from the Actinobacteria to the Proteobacteria possess the capability for ethanolamine metabolism, which does not require eut genes (Brian et al., 2015; Gerlt et al., 2016). The most likely steps of the ethanolamine utilization pathway in *S. coelicolor* may be similar to the pathway described in studies of Brian et al., 2015 and Gerlt et al., 2016 as well as in detail in *C. salexigens* by Gerlt et al., 2016. After the glutamylation of ethanolamine by GlnA4 (SCO1613), a predicted gammaglutamylethanolamine dehydrogenase (SCO1611) may be required, producing gamma-glutamylacetaldehyde. In the next step of the pathway, a predicted gammaglutamylaldehyde dehydrogenase (SCO1612) may be involved, producing gammaglutamylglycine. The last step of the pathway may require a predicted gammaglutamylglycine amidohydrolase (SCO1615). The pathways may end in the production of glycine and glutamate. Further studies of SCO1611, SCO1612 and SCO1615 are required to determine their functionality in the ammaglutamylation pathway of ethanolamine in *S. coelicolor* (Krysenko et al., 2019, 2021).

1.1.3. Nitrogen Assimilation in Actinomycetales – Transcriptional Regulation

1.1.3.1. Regulation of Central Pathways

Nitrogen assimilation in Enterobacteriaceae is regulated by the Ntr system, however, most Actinomycetales possess the global transcriptional regulator GlnR for the same purpose (Amon et al., 2008; Tiffert et al., 2008). Homodimerization of this regulator is necessary for DNA binding and thus for regulation. In the Ntr system, phosphorylation of the conserved Asp residue by NtrB ensures NtrC dimerization. Since a GlnR with a conserved Asp residue in the N-terminal receiver domain was found in Actinomycetales, but no kinase phosphorylating this Asp residue, GlnR was long considered an "orphan" regulator. Then it was discovered that the conserved Asp residue is not phosphorylated and that the activating GlnR dimerization in A. mediterranei is caused by the ionic interaction of unphosphorylated Asp50 with the conserved Arg52 and Thr9 (Lin et al., 2014). Due to the lack of phosphorylation of Asp, GlnR is considered an atypical OmpR-like regulator. The receiver domain of one GlnR molecule forms an α4-β5-α5 surface, through which interaction with the receiver domain of the second GlnR molecule takes place (Lin et al., 2014). More than 15 genes are under the control of GlnR, most of which regulate nitrogen metabolism (Tiffert et al., 2008). GlnR exerts its activating effect on gene transcription under nitrogen deficiency by binding to the promoter regions of the regulated genes, thereby altering transcription (Tiffert et al., 2011). Thus, the *amtB* operon in *S. coelicolor* is controlled by GlnR activation (Fink et al., 2002), with a correlation between transcription and the amount of nitrogen found (Nolden et al., 2001; Fink et al., 2002). This again differs from the regulation in *E. coli*, where *glnD* expression has been described as constitutive (van Heeswijk et al., 1996). In addition to GlnR, Actinomycetes also contain GlnRII, which is less common in bacteria and has not been studied as extensively as GlnR. Like GlnR, GlnRII plays a role a regulatory role in the

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genes of N metabolism. However, so far, GlnRII has only been shown to affect the genes *glnA*, *amtB*, and *glnII* (Fink et al., 2002).

Another transcriptional regulator of N metabolism that is widespread among Actinomycetes is AmtR. In Corynebacteria, AmtR, a regulator of the TetR family that acts as a repressor of gene expression (Jakoby et al., 2000), replaces the GlnR regulator. In N excess, AmtR is bound to DNA, thus preventing the expression of key genes involved in N metabolism, such as *glnA*, *gltB*, and the *amtB* operon (Jakoby et al., 2000; Nolden et al., 2001; Beckers et al., 2005). In N deficiency, an adenylated GlnK gene product binds to AmtR and leads to the dissociation of the repressor from the DNA, allowing the released genes to be transcribed (Beckers et al., 2005). It was recently discovered that some members of the Corynebacterineae and *Streptomyces*, which possess a GlnR regulator, also possess an AmtR protein that shows low homology to the AmtR in Corynebacteria (Amon et al., 2008). Even though TetR regulators possess a highly conserved DNA-binding domain, AmtR regulators exhibit individual recognition features in the amino acid sequence (Muhl et al., 2009). Subsequent investigation of these AmtR regulators revealed that GlnR plays the main role in the regulation of nitrogen assimilation, while AmtR regulates only a subset of genes and its regulation is GlnR-dependent (Jessberger et al., 2013). This AmtR regulon includes genes for amidase, urea carboxylase, and amino acid permease (Jessberger et al., 2013).

1.1.3.2. Regulation of Catabolism of Amines in Actinomycetes

In *S. coelicolor*, the global regulator of the nitrogen metabolism GlnR can undergo post-translational modifications that affect its binding affinity to DNA (Amin et al., 2016). It was shown that the phosphorylation of Ser/Thr occurs under N-excess conditions. In agreement with these results, lack of phosphorylation on the Asp50 and lack of any Ser/Thr phosphorylation in GlnR isolated from *S. coelicolor* grown under N-limiting conditions was also demonstrated (Lin et al., 2014). Phopshorylation and acetylation were shown to influence the DNA-binding affinity of GlnR (Amin et al., 2016). GlnR regulates the glutamine synthetase encoding genes *glnA* and *glnII* at transcriptional level in dependence of N-conditions. However, no binding GlnR in promoter regions of *glnA2*, *glnA3* and *glnA4* were reported (Fink et al., 2002; Rexer et al., 2006). However, the acetylated version of GlnR binds better to the *glnA2* promoter region (Krysenko et al., 2022). Interestingly, studies in the actynomycete Saccharopolyspora erythraea revealed that GlnR activates the expression of *glnA3* (*SACE_3095*) (Yao et al., 2014).

In the genome of *S. coelicolor*, the *sco5656* (*epuRII*) gene is localized close to the genes that encode predicted enzymes of polyamine utilization pathway (Krysenko et al., 2022) and is annotated as putative regulator (Bentley et al., 2002). The expression of the *epuRII* gene in presence of putrescine, cadaverine and spermidine was enhanced (Krysenko et al., 2017). EMSA analysis of regulatory targets of EpuRII revealed hints towards a complex regulation of several polyamine associated genes. These include *glnA3*, as well as sco5676 encoding a putative homolog of the 4-amino-butyrate aminotransferase GabT of *E. coli* K12 and *sco5977* encoding a putative polyamine antiporter. The tests with EpuRII resulted in eight positive hits for EpuRII-interacting promoter sequences. The following genes seem to be regulated by EpuRII: *glnA3*, *sco5676*, coding for a putative homologue of the 4-amino-butyrate aminotransferase GabT, *sco5977* encoding a putative polyamine antiporter, and *sco6960* with unknown function (Krysenko et al., 2022).

There are two known regulatory mechanisms of ethanolamine utilization genes: the EutR system and the EutVW system. Regulation of ethanolamine utilization genes was well studied in *S. typhimurium*, *E. coli* and *E. faecalis*. In *S. coelicolor*, the *sco1614* (*epuRI*) gene was annotated as putative regulator in the genome of *S. coelicolor* that is localized close to the gene *glnA4* (*sco1613*) (Bentley et al., 2002). EMSA analysis revealed *glnA4* gene as a potential target of EpuRI. Other genes located downstream of *glnA4*, namely *sco1612*, *sco1611* and *sco1610*, encode predicted enzymes of the ethanolamine utilization pathway and are organized in one putative operon together with *glnA4*. Thus, these genes may be transcribed together with *glnA4*. The role of EpuRI as a negative transcriptional regulator of the ethanolamine utilization associated genes was shown in a

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transcriptional analysis (Krysenko et al., 2019). Interestingly, in preliminary EMSA analysis also interactions of EpuRI with promoter sequences of *sco5652* and with the adjacent operon including *sco5654* were observed. The *sco5652* gene encodes a protein of unknown function, *sco5654* encodes a putative ABC transporter. EpuRI demonstrated interactions with the promoter region of *sco5657* (encoding a putative aldehyde dehydrogenase), *sco1616* (a putative regulator) and the promoter sequence of *epuRI* itself. Regulatory network that involves EpuRI seems to be complex (Krysenko et al., 2019, 2022).

3. Conclusions and Future Perspectives

Nitrogen metabolism is essential for bacterial survival under nutrient limitation conditions in competitive ecological niches. Bacteria have developed complex metabolic networks and regulatory machinery to control intracellular pools of nitrogen. Nitrogen metabolism comprising transport, biosynthesis, utilization and regulation is a central part of bacterial primary metabolism, ensuring supply of building blocks for biomolecules and biomass generation. Catabolism of nitrogen-containing compounds, like ammonium, nitrate, amino acids, amino sugars, urea and amines ensures bacterial survival and pathogenicity. In this regard, metabolism of amines has been investigated in several human pathogenic bacteria in connection to infection process. Studies in pathogenic bacterial species including *Salmonella typhimurium*, *Brucella abortus*, *Mycobacterium tuberculosis*, *Chlamydia pneumoniae*, *Legionella pneumophila*, *Listeria monocytogenes*, and some others prove the crucial role of polyamines for their proliferation. The interconnection between the biosynthesis, uptake, and assimilation of amines remains crucial to find new therapeutic drug targets (Krysenko & Makhoba, 2025). Most human pathogens rely not only on polyamine biosynthesis, but also on polyamine detoxification to proliferate and maintain infection, targeting metabolism of amines can extend the options for combating bacterial infections (Krysenko et al., 2023, 2025). There is an urgent need to find new anti-bacterial drugs with novel modes of action that would be efficient on bacterial infections, especially in the light of emergence of resistances. Investigation of the pathways in nitrogen metabolism required for bacterial growth, survival, and pathogenicity provide new drug target candidates for development of more effective agents.

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