Enzymes and pathways of polyamine breakdown in microorganisms

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1: SUMMARY

The information currently available on the breakdown of spermidine and putrescine by microorganisms is reviewed. Two major metabolic routes have been described, one for the free bases via Δ1-pyrroline (4-aminobutyraldehyde), the other via N-acetyl derivatives. In both pathways oxidases or aminotransferases are the key enzymes in removing the nitrogen atoms. The two routes converge at 4-aminobutyrate, which is then metabolized via succinate. The degradation of putrescine in Escherichia coli has been well characterized at both genetic and biochemical levels, but for other bacteria much less information is available. The C3 moiety of spermidine is broken down via β-alanine, but the metabolism of this compound and its precursors is poorly understood. In yeasts, a catabolic route for spermidine and putrescine via N-acetyl derivatives has been described in Candida boidinii, and the evidence for its occurrence in other species is reviewed. Except for the terminal step of this pathway, the same group of enzymes can metabolize both the C3 and C4 moieties of spermidine. It's likely that other routes of polyamine catabolism also exist in both bacteria and yeasts.

2. INTRODUCTION

Polyamines are polycations found in most cells with an important, if very poorly understood biological role. Despite the proved necessity of their presence for normal cellular growth, their molecular functions remain unknown [1]. The three most important polyamines are 1,4-diaminobutane (putrescine), 1,8-diamino-4-azaoctane (spermidine) and 1,12-diamino-4,9-diazadodecane (spermine) (for the structures of spermidine and putrescine, see Fig. 3).

The carbon chains of the polyamines arise biosynthetically from amino acids, the C4 fragment from ornithine and the C3 fragment(s) from...
methionine [1]. The biosynthetic pathway of polyamines and many of their biological effects were demonstrated in microorganisms long before the systems were defined in animals [1], but much less is known about their breakdown in microorganisms [2]. This review covers the catabolic routes involving the removal of the nitrogen atoms of polyamines, and traces the fate of the carbon skeletons as far as succinate and malonate semialdehyde.

The discussion that follows mainly considers spermidine and putrescine. Not only is spermine quantitatively less important [1], it can be metabolized by exactly the same pathway and enzymes as spermidine, the only difference being that its breakdown generates two C\textsubscript{3} fragments rather than one. Similarly, the unusual polyamine homologues and analogues found in thermophilic bacteria [3] may also be degraded by the same metabolic routes as the more usual polyamines.

3. TWO POSSIBLE DEGRADATIVE ROUTES?

The ability of microorganisms to degrade polyamines has never been the subject of a systematic survey. Consequently, there is a lack of basic information about the breakdown routes and the types of enzyme involved. The most useful information has come from experiments in which the polyamine (or degradation products) is supplied to microorganisms as sole nitrogen (N) source or as sole carbon (C) and N source (see section 4.1). From this type of experiment, it seems probable that there are at least two different degradative routes for spermidine and putrescine, one involving the free bases, the other involving N-acetyl derivatives (Fig. 1). Both routes converge on 4-aminobutyrate, and in all organisms so far characterized, polyamine degradation is closely associated with the metabolism of 4-aminobutyrate. It is common to all routes that some of the nitrogen atoms are removed by oxidase mechanisms and others by aminotransferase mechanisms, with an oxidative or dehydrogenative cleavage at the spermidine secondary amino group.

Other derivatives of polyamines, such as N-glutathionylspermidine [4], whose function remains unknown, may be part of as yet uncharacterized metabolic routes for polyamine breakdown.

4. THE NON-ACETYLATED ROUTE

4.1. Breakdown of spermine and spermidine

The few reports that existed until recently on the degradation of spermine and spermidine suggested that spermine was broken down via spermidine, 4-aminobutyrate, 1,3-diaminopropane and \(\beta\)-alanine [5]. The fate of these products, particularly the C\textsubscript{3} moiety of spermidine, remains unknown in many organisms.

The only degradative enzyme for free spermidine to have been characterized in detail is the flavohaemoprotein, nicotinamide nucleotide-
independent spermidine dehydrogenase (EC 1.5.99.6) catalysing reaction:

\[
\begin{align*}
\text{NH}_2^+ - [\text{CH}_2]_3 - \text{NH}_2^+ - [\text{CH}_2]_2 - \text{NH}_2^+ \\
\rightarrow \text{CH}_2 - \text{C}^+ + \text{CH}_2 - \text{CN}^+ + 3\text{H}^+ + 2\text{e} \quad (1)
\end{align*}
\]

This enzyme has been purified from *Serratia marcescens* [6] and it forms the basis of enzymic methods for the estimation of spermidine [7]. Recent work by Hisano et al. [8] on the ability of bacteria to use spermidine as a growth substrate is the first attempt to examine the distribution of spermidine dehydrogenase in a range of microorganisms. Of 24 bacterial strains screened, only four species showed substantial growth on spermidine, although several others showed poorer growth. *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Flavobacterium dormitator* were able to use spermidine as either a C source (when ammonia was the N source) or as a N source (when glucose was the C source). Of these, the first three contained relatively high levels of spermidine dehydrogenase, measured with ferricyanide as the electron acceptor. The enzyme from *Citrobacter freundii* (which was inducible by spermidine) and the constitutive enzyme of *Pseudomonas aeruginosa* were purified and shown to consist of a single polypeptide chain of $M_r$ 63,000 and an absorption maximum of 430 nm. These properties are very similar to those described for the enzyme from *Serratia marcescens* [6], except that the latter had an $M_r$ of 76,000. *Citrobacter freundii* was unusual in having two isoenzymes with identical $M_r$ values and amino terminal sequences but being separable on hydroxyapatite [8]. Spermine, spermidine and N-glutathionylspermidine were substrates (see section 5.1). Although it may be presumed that the product, $\Delta^1$-pyrroline, is metabolized via

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![Fig. 2. The breakdown of arginine, ornithine and putrescine in *Escherichia coli*. The genes for each enzymic step are indicated above the arrows. Enzymes: (1) arginine decarboxylase, (2) agmatine ureohydrolase, (3) ornithine decarboxylase, (4) diamine aminotransferase, (5) $\Delta^1$-pyrroline dehydrogenase, (6) 4-aminobutyrate aminotransferase, (7) succinate semialdehyde dehydrogenase (NADP-linked).](image-url)
4-aminobutyrate and the 1,3-diaminopropane via β-alanine, no definitive evidence has yet been obtained.

Less is known about an enzyme from a *Pseudomonas* species grown on spermidine as sole C and N source. This may be a polyamine oxidase because it degrades spermidine to 3-aminopropionaldehyde and putrescine [9]. If so, it is the first demonstration of a polyamine oxidase in bacteria (see section 5.2).

4.2. Breakdown of putrescine

Putrescine is the only polyamine whose breakdown has been studied systematically in bacteria [10]. Although wild-type *Escherichia coli* K-12 does not use either putrescine or 4-aminobutyrate as N source [11], wild-type *Escherichia coli* B can use 4-aminobutyrate as sole N source [12] and mutants of *Escherichia coli* K-12 that use 4-aminobutyrate as N source can be selected [11]. From these strains further mutants capable of using putrescine as sole C and N source (*Escherichia coli* B) [13], 4-aminobutyrate as sole C and N source (*Escherichia coli* K-12) [11] and putrescine as sole C and N source (*Escherichia coli* K-12) [10], can be selected. Putrescine breakdown in *Escherichia coli* (Fig. 2) has been well documented both genetically [14,15] and biochemically [10].

The conversion of putrescine into Δ1-pyrroline (4-aminobutyraldehyde) in bacteria proceeds either via an aminotransferase or via an oxidase.

In *Escherichia coli* putrescine is converted non-oxidatively into Δ1-pyrroline by the action of diamine: 2-oxoglutarate aminotransferase (EC 2.6.1.29):

\[
\text{NH}_3^+ - [\text{CH}_2]_4 - \text{NH}_3^+ + 2\text{-Oxoglutarate} \rightarrow \text{L-Glutamate} + \text{CH}_2 - \text{CH}_2 + \text{H}_3\text{O}^+ + \text{CH}_2 - \text{N} \quad \text{CH} + \text{H}_2\text{O}_2
\]  

(2)

The enzyme has been partially purified [16]. It has a pH optimum between 9 and 10, contains pyridoxal phosphate and in addition to putrescine shows activity with 1,5-diaminopentane, 1,7-diaminohexane and 4-aminobutyrate but not with 1,3-diaminopropane.

Putrescine oxidase (EC 1.4.3.10) catalyses the direct oxidation of putrescine by the following reaction:

\[
\text{NH}_3^+ - [\text{CH}_2]_4 - \text{NH}_3^+ + \text{O}_2 \rightarrow \text{CH}_2 - \text{CH}_2 + \text{NH}_4^+ + \text{H}^+ + \text{CH}_2 - \text{N} \quad \text{CH} + \text{H}_2\text{O}_2
\]  

(3)

It is a flavoprotein containing one FAD per mol [17] and has two different subunits, \( M_r \) values 43000 and 51000 [18]. The enzyme, which has been purified only from *Micrococcus rubens* [18], can be used to estimate putrescine enzymically by several different methods [19,20], although it also oxidizes spermidine slowly to Δ1-pyrroline, 1,3-diaminopropane and \( \text{H}_2\text{O}_2 \) [21]. There is very little information as to the conditions in which this enzyme is expressed or whether it occurs in other organisms.

In addition to putrescine oxidase, most bacterial diamine oxidases (EC 1.4.3.6) are able to degrade putrescine giving the same products, but they have different prosthetic groups, copper and possibly topaquinone instead of FAD [22], and their possible role in putrescine breakdown has not been examined.

The distribution of the oxidase and aminotransferase routes was investigated by Michaels and Kim [23] using organisms grown on putrescine either as sole N source or as sole C and N source. Of the species examined, six contained both types of enzyme (*Bacillus cereus*, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Chromobacterium violaceum* and *Phytomonas fasciata*) and four had only the aminotransferase (*Aerobacter aerogenes*, *Escherichia coli*, *Serratia marcescens* and *Staphylococcus aureus*). One (*Haemophilus parainfluenzae*) had only diamine oxidase, and others exhibited neither enzyme activity. These organisms required yeast extract for growth and were probably not metabolizing the putrescine.

In addition, putrescine is a reported substrate for 4-aminobutyrate aminotransferase ([24], see
below) and \(\omega\)-amino acid aminotransferase ([25], see below), but it seems improbable that these activities are physiologically significant.

4.3. Conversion of \(\Delta^1\)-pyrroline to succinate semialdehyde

The positions of the genes encoding two key enzymes of the non-acetylated route of putrescine breakdown (see Fig. 2) were mapped by Shaibe et al. [14] and the diamine aminotransferase (\(par\)) and \(\Delta^1\)-pyrroline dehydrogenase (\(prr\)) genes were located at 89 and 30 min respectively on the \textit{Escherichia coli} K-12 chromosome. Mutants defective in either of these enzymes had growth yields for putrescine of only about 10% of the wild-type and had about 10% residual enzyme activity [14]. In addition to being involved in polyamine breakdown, this pathway is also used in \textit{Escherichia coli} for the breakdown of arginine, agmatine and ornithine (Fig. 2).

The two enzymes have been characterized in the mutants of \textit{Escherichia coli} K-12 that use putrescine as C source as well as N source [10]. \(\Delta^1\)-Pyrroline (4-aminobutyraldehyde) dehydrogenase (EC 1.2.1.19) has also been purified from a \textit{Pseudomonas} species grown on spermidine or putrescine as a sole C and N source [26,27] and from \textit{Pseudomonas putida} grown on putrescine or arginine [28]. The product of the reaction is 4-aminobutyrate, which is metabolized via succinate semialdehyde in both \textit{Escherichia coli} [11] and \textit{Pseudomonas fluorescens} [29].

4-Aminobutyrate aminotransferase (EC 2.6.1.19), diamine aminotransferase, \(\Delta^1\)-pyrroline dehydrogenase and succinate semialdehyde dehydrogenase are regulated in \textit{Escherichia coli} K-12 by cAMP-independent catabolite repression [15]. The use of putrescine as N source is accompanied by elevated levels of all these enzymes. 4-Aminobutyrate aminotransferase has been purified from \textit{Pseudomonas fluorescens} by Scott and Jakoby [29]. In \textit{Escherichia coli} this enzyme is encoded by the \(gabT\) gene and the reaction product is succinate semialdehyde. No other \(\omega\)-amino acid (including \(\beta\)-alanine) was used as a substrate. The enzyme contains pyridoxal phosphate and has optimum activity between pH 8.5 and 9.

In \textit{Pseudomonas fluorescens} the \(\Delta^1\)-pyrroline dehydrogenase and 4-aminobutyrate aminotransferase activities were also elevated in cells using putrescine as the N source [30].

4.4. Oxidation of succinate semialdehyde

The final step in the putrescine degradation pathway is the oxidation of succinate semialdehyde to succinate, catalysed by succinate semialdehyde dehydrogenase. \textit{Escherichia coli} B and K-12 [12,31] and \textit{Pseudomonas putida} [32] contain two such enzymes, while \textit{Klebsiella aerogenes} contains only one [33]. In \textit{Escherichia coli}, both succinate semialdehyde dehydrogenases (one NAD-specific, \(M_r\) 97 000, EC 1.2.1.24; the other NADP-specific, \(M_r\) 200 000, EC 1.2.1.16) are elevated in cells grown on 4-aminobutyrate, although only the latter is induced by 4-aminobutyrate. The former is induced by succinate semialdehyde [12]. The use of 4-aminobutyrate as N source induces high levels of 4-aminobutyrate aminotransferase [31]. In \textit{Pseudomonas putida} putrescine induces elevated levels of the NADP-dependent succinate semialdehyde dehydrogenase, \(\Delta^1\)-pyrroline dehydrogenase and 4-aminobutyrate aminotransferase [32]. In another \textit{Pseudomonas} species, hybrid NAD-dependent succinate semialdehyde dehydrogenase isoenzymes have been described which are also active with aminoaaldehydes [34]. Close immunological relationships have also been shown between succinate semialdehyde dehydrogenase and aminoaldehyde dehydrogenase activity [35]. This highlights the close association between polyamine breakdown and 4-aminobutyrate breakdown.

4.5. Metabolism of diaminopropane

1,3-Diaminopropane (see Fig. 1) is not a substrate for putrescine oxidase or diamine: 2-oxoglutarate aminotransferase [36], but recent work has demonstrated that in a coryneform bacterium grown on diaminopropane as sole C and N source, there is a pyruvate-dependent aminotransferase that shows high activity towards diaminopropane (and somewhat lower activity with other diamines) [36]. The presumed reaction product, 3-aminopropionaldehyde, is a substrate for aminoaldehyde dehydrogenase [26], yielding \(\beta\)-alanine.
5. THE N-ACETYL ROUTE

5.1. N-Acetylpolyamines

Until recently, the route via Δ1-pyrroline seemed to be the only accepted pathway for polyamine degradation in microorganisms, despite the isolation in 1960 of monoacetylpolyamine and both isomers of monoacetylspermidine from cells of *Escherichia coli* grown on glucose-ammonium salts medium [37]. Their concentrations increased markedly if the culture was kept at 4–6°C [38]. An acetyltransferase leading to the formation exclusively of N-acetylspermidine has been described in extracts of *Escherichia coli* [39] but apparently not yet purified. Since the enzyme was inactive with putrescine, it is not clear how acetylputrescine could be formed in this organism. It seems possible that there may yet be an undetected putrescine acetyltransferase in *Escherichia coli*, because the growth medium of Matsui et al. [39] was not supplemented with polyamines. Recent work [40] has shown that added extracellular acetylpolyamines can be taken up by cells of *Escherichia coli* by the spermidine transport mechanism.

Enzymes catalysing the deacetylation of acetylputrescine have been detected in cells of various microorganisms, but the physiological role of these enzymes is not yet apparent [41,42]. In *Escherichia coli*, acetylpolyamines are inactive in most in vitro systems including cell-free protein-synthesizing systems [43], in which the parent polyamines show activity.

*N*1-Acetylsperrmine did not stimulate the growth of polyamine-requiring mutants of *Escherichia coli* although it entered the cells [43]. *N*-Acetyl derivatives of other polyamines did stimulate growth, but only insofar as they were deacetylated in the cell. The current view [43] on the role of *N*-acetylpolyamines in *Escherichia coli* is that acetylation leading to loss of activity may provide a mechanism for regulating the intracellular level of the parent polyamines when excess amounts are present in the cell. The observed loss of activity of polyamines on acetylation, however, is also compatible with the suggestion now being made that acetylation is the first step in their degradation. Acetylated polyamine metabolism in bacteria needs further investigation [42] to see whether in fact acetyl derivatives may repre-

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Table 1

<table>
<thead>
<tr>
<th>Yeast species</th>
<th>Oxidation of spermidine</th>
<th>Oxidation of free putrescine</th>
<th>Oxidation of acetylputrescine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary amino group attacked [59]</td>
<td>Secondary amino group attacked [46]</td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida utilis</em></td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><em>Candida steatolytica</em></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Pichia pastoris</em></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

| Group II      |                         |                               |                               |
| *Candida boidinii* | No            | Yes                            | No                            |
| *Candida nagoyensis* | No            | Yes                            | No                            |
| *Hansenula polymorpha* | No           | Yes                            | No                            |
| *Trichosporon melibiosaceum* | No       | Yes                            | NT                            |

| Group III     |                         |                               |                               |
| *Sporopachydenia cereana* | Yes         | Yes                            | Yes                           |

Extracts of cells grown on spermidine or putrescine as sole nitrogen source were tested for amine oxidase activity. The reference cited give the assay methods used.

*NT*, not tested.
sent a new catabolic route and not a dead-end, as has often been assumed. It is possible for example that in some bacteria, putrescine breakdown may occur by an N-acetyl route, as was first proposed for mammalian brain tissue by Seiler and Al-Therib [44].

Bacterial enzymes active with the free polyamines need to be tested with the corresponding N-acetyl derivatives, as has been done with polyamine oxidase in eukaryotes (see section 5.2). Spermidine dehydrogenase from Citrobacter freundii [8] shows activity with N-glutathionyl-spermidine (giving rise to N-glutathionyl-diaminopropionate) [45], and it is possible that this enzyme might also show activity with N-acetyl-spermidine(s).

In yeasts, polyamine breakdown had not been studied until recently. This is because Saccharomyces cerevisiae cannot use amines of any kind as N source for growth. However, it is now known that many other yeasts can indeed use spermidine and putrescine as the N source for growth [46], and some yeasts that can use putrescine as sole C source have also been described [47].

Examination of a limited number of yeast species (Table 1) has suggested that there are two groups: those that can attack polyamines by oxidation at the primary amino group (eqn. 4):

\[
\begin{align*}
\text{NH}_3^+ - [\text{CH}_2]_3 - \text{NH}_2^+ - [\text{CH}_2]_3 - \text{NH}_3^+ & \\
\xrightarrow{\text{O}_2} & \text{NH}_3^+ - [\text{CH}_2]_3 - \text{NH}_2^+ - [\text{CH}_2]_3 - \text{CHO} + \text{NH}_4^+ + \text{H}_2\text{O}_2 \\
\xrightarrow{\text{O}_2} & \text{OHC}-[\text{CH}_2]_2 - \text{NH}_2^+ - [\text{CH}_2]_3 - \text{CHO} + \text{NH}_4^+ + \text{H}_2\text{O}_2
\end{align*}
\]

and those which cleave the molecule by oxidation at the secondary amino groups (probably after first acetylating at least one of the primary amino groups, see sections 5.2 and 5.3). The first group of yeasts in Table 1 has not yet been examined properly. It seems likely from cell-free enzyme activities, that they oxidize polyamines at the primary amino groups giving rise to dialdehydes (eqn. 4). If so, a new breakdown route involving dialdehydes may exist. These compounds are known to be cytotoxic [48].

<table>
<thead>
<tr>
<th></th>
<th>Candida boidinii</th>
<th>Penicillium chrysogenum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of enzyme</td>
<td>Peroxisomal</td>
<td>Extracellular</td>
</tr>
<tr>
<td>pH optimum for spermidine oxidation</td>
<td>10</td>
<td>4 (small peak at 9.5)</td>
</tr>
<tr>
<td>Molecular mass</td>
<td>80–110 kDa</td>
<td>135 kDa</td>
</tr>
<tr>
<td></td>
<td>(K_m) ((\mu)M)</td>
<td>(V_{max}) (\mu)M</td>
</tr>
<tr>
<td>Spermidine</td>
<td>170</td>
<td>1.2</td>
</tr>
<tr>
<td>(N^1)-Acetyl-spermidine</td>
<td>30</td>
<td>34.0</td>
</tr>
<tr>
<td>(N^8)-Acetyl-spermidine</td>
<td>320</td>
<td>10.3</td>
</tr>
</tbody>
</table>

5.2. Polyamine oxidase

The yeasts of group II in Table 1 cleave polyamines at the secondary amino group using a polyamine oxidase (FAD-dependent) (EC 1.5.3.-) [46] similar to the peroxisomal enzyme found in mammals [49]:

\[
\begin{align*}
\text{R-NH}_2 - [\text{CH}_2]_3 - \text{NH}_2^+ - [\text{CH}_2]_2 - \text{NH}_2^+ & \\
+ \text{O}_2 + \text{H}_2\text{O} & \\
\rightarrow & \text{R-NH}_2 - [\text{CH}_2]_2 - \text{CHO} + \text{NH}_3^+ - [\text{CH}_2]_4 - \text{NH}_3^+ + \text{H}_2\text{O}_2 \\
& (R=\text{H} \text{ or } \text{CH}_3\text{CO})
\end{align*}
\]

Such an enzyme also occurs in filamentous fungi [50]. It is important to note that polyamine oxidases are active with N-acetyl-polyamines as well as with the free bases. However, the polyamine oxidase that was partially purified from Candida boidinii [46] differed markedly in its properties from the one in filamentous fungi (Table 2). The data for the \(K_m\) values and relative oxidation rates suggest that the Penicillium chrysogenum enzyme functions in the oxidation of the free spermine and spermidine bases [51] while the kinetic data for the enzyme from Candida boidinii [46,54] suggest that acetylated derivatives are its physiological substrates. This latter mechanism is
in keeping with the postulated role of polyamine oxidase in mammalian tissue [53].

Potent inhibitors of polyamine oxidase are known [52,53], but they have been found to be of no value for detecting the presence of the N-acetyl breakdown route because they are ineffective with whole yeast cells (A. Robertson and P.J. Large, unpublished observations).

5.3. N-Acetylation of polyamines

If acetylspermidines are the physiological substrates of polyamine oxidase there should be enzymes which acetylate spermidine. Such an enzyme, spermidine acetyltransferase (EC 2.3.1.57), has now been found in *Candida boidinii* [54]. Its instability prevented purification to homogeneity, but evidence was presented that the same enzyme catalysed the acetyl-CoA-dependent acetylation of spermine, spermidine and putrescine, but unlike the mammalian cytosolic enzyme (EC 2.3.1.-) [55] which catalyses exclusively the formation of N¹-acetylsperrmidine, the yeast enzyme formed both N¹- and N⁸-acetylsperrmidine in a ratio of 50:45. This agrees with the specificity of the yeast polyamine oxidase for N¹- and N⁸-acetylsperrmidine derivatives, whereas the rat liver

![Diagram](https://example.com/diagram.png)

**Fig. 3.** The N-acetyl route for the breakdown of spermidine in *Candida boidinii* [65] (reproduced by permission of John Wiley & Sons). The intermediates are: I, spermidine; II, N¹-acetylsperrmidine; III, N⁸-acetylsperrmidine; IV, 3-acetamidopropionaldehyde; V, putrescine; VI, 4-acetamidobutyraldehyde; VII, 1,3-diaminopropane; VIII, acetylpberrscine; IX, 4-acetamidopropylamine; X, 3-acetamidopropionate; XI, β-alanine; XII, 4-acetamidobutyrate; XIII, 4-aminobutyrate; XIV, malonate semialdehyde; XV, succinate semialdehyde. Enzymes: (1) diamine acetyltransferase; (2) polyamine oxidase; (3) acetylpberrscine oxidase; (4) acetamidoaldehyde dehydrogenase; (5) 4-acetamidobutyrate deacetylase; (6) 4-aminobutyrate and β-alanine aminotransferases.
Table 3
Specific activities of enzymes of putrescine breakdown in *Candida boidinii* grown on different nitrogen sources ([63], reproduced by permission of John Wiley and Sons).

<table>
<thead>
<tr>
<th>Enzyme specific activity (nmol min⁻¹ (mg protein)⁻¹)</th>
<th>Nitrogen source for growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spermidine</td>
</tr>
<tr>
<td>Diamine acetyltransferase</td>
<td>293</td>
</tr>
<tr>
<td>Acetylputrescine oxidase</td>
<td>88</td>
</tr>
<tr>
<td>Catalase (× 10⁻³)</td>
<td>122</td>
</tr>
<tr>
<td>Acetamidoaldehyde dehydrogenase A</td>
<td>328</td>
</tr>
<tr>
<td>Acetamidoaldehyde dehydrogenase B</td>
<td></td>
</tr>
<tr>
<td>(acetamidopropionaldehyde as substrate)</td>
<td>62</td>
</tr>
<tr>
<td>(acetamidobutyraldehyde as substrate)</td>
<td>49</td>
</tr>
<tr>
<td>Acetamidoacetyltransferase</td>
<td>31</td>
</tr>
<tr>
<td>4-Aminobutyrate aminotransferase</td>
<td>122</td>
</tr>
<tr>
<td>β-Alanine: 2-oxoglutarate aminotransferase</td>
<td>5</td>
</tr>
</tbody>
</table>

Polyamine oxidase will only catalyse the breakdown of \(N^1\)-acetylspermidine [56,57]. The specificity of the *Candida* enzyme also differs from the calf liver nuclear enzyme, histone acetyltransferase (EC 2.3.1.48) which forms 85% \(N^2\)-acetylspermidine and only 15% \(N^1\)-acetylspermidine [58]. Polyamine oxidase and diamine acetyltransferase were located respectively in the peroxisomes and the mitochondria of *Candida boidinii* [46,54]. The combined effects of the

Table 4
Distribution of enzymes of the \(N\)-acetyl pathway in yeasts grown on putrescine (unpublished data of A. Robertson and P.J. Large).

<table>
<thead>
<tr>
<th>Nitrogen source:</th>
<th><em>Candida boidinii</em></th>
<th><em>Candida nagoyaensis</em></th>
<th><em>Candida utilis</em></th>
<th>Hansenula polymorpha</th>
<th>Sporopachydermia cereana</th>
<th>Pichia pastoris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Putrescine</td>
<td>Ammonia</td>
<td>Putrescine</td>
<td>Putrescine</td>
<td>Putrescine</td>
<td>Putrescine</td>
</tr>
<tr>
<td>Polyamine oxidase</td>
<td>53</td>
<td>0</td>
<td>27</td>
<td>0</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Catalase (× 10⁻²)</td>
<td>132</td>
<td>21</td>
<td>600</td>
<td>64</td>
<td>60</td>
<td>209</td>
</tr>
<tr>
<td>Diamine acetyltransferase</td>
<td>31</td>
<td>0</td>
<td>39</td>
<td>41</td>
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<td>13</td>
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<tr>
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<td>89</td>
<td>18</td>
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</table>

Cultures were grown on 55 mM glucose with 5 mM putrescine or 30 mM ammonia as nitrogen source [46]. For details of enzyme assays, see [46,54,60,61,63]. NAD was the electron acceptor in the dehydrogenase assays.
acetyltransferase and polyamine oxidase are to generate a mixture of four products (Fig. 3), putrescine, 1,3-diaminopropane, 4-acetamidobutyraldehyde and 3-acetamidopropionaldehyde. Further metabolism then requires acetylation of the two free bases, and indeed the Candida boidinii acetyltransferase was found to acetylate 1,3-diaminopropane as well as putrescine. The acetylated route thus conveniently allows both the C₃ and the C₄ breakdown products of spermidine to be metabolized by the same enzymes, with the exception of the final transamination steps of 4-aminobutyrate and β-alanine.

5.4. Conversion of N-acetylpolyamines to 4-aminobutyrate

The two products acetylputrescine and acetyl-1,3-diaminopropane (3-acetamidopropylamine) are substrates for one of the two broad specificity primary-amine oxidases of Candida boidinii, an enzyme (EC 1.4.3.6) previously purified under the name benzylamine oxidase [59], but now more accurately termed an acetylputrescine oxidase [61] (Tables 3 and 4). Both of the two acetylated diamines are good substrates for this enzyme, while the free bases are not, and 3-acetamidopropionaldehyde and 4-acetamidobutyraldehyde are formed (Fig. 3).

Two aldehyde dehydrogenases that would catalyse the oxidation of both of these two products were found in Candida boidinii [60]. One dehydrogenase (A) was completely inhibited by 10 mM chloral hydrate, was essentially constitutive and was shown to be mitochondrial in its location. The other (B) was greatly increased in activity in cells grown on putrescine and spermidine (Table 3) and was cytosolic in its location. Enzyme B was considered to be the enzyme most likely to be involved in polyamine oxidation. Both enzymes were active with both NAD and NADP, but enzyme B had a much higher affinity for the two acetamidoaldehydes.

The products of oxidation of the two aldehydes are the corresponding acetamido carboxylic acids, N-acetyl-β-alanine and 4-acetamidobutyrate. Before these can enter the final degradative stages, they must be deacetylated. An enzyme was found in Candida boidinii that would catalyse the deacetylation of both 4-acetamidobutyrate and N-acetyl-β-alanine [61]:

\[
\text{CH}_3\text{CO-NH-}[\text{CH}_2]_n\text{-COO}^- + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COO}^- + \text{NH}_3^+ + [\text{CH}_2]_n\text{-COO}^- \quad (6)
\]

The enzyme was present in cells grown on spermidine, putrescine and 4-acetamidobutyrate as sole N source, but not in cells grown on ammonia, lysine or 4-aminobutyrate (Table 3).

The enzyme, acetamidobutyrate deacetylase (EC 3.5.1.63) was partially purified 250-fold from Candida boidinii. It had a molecular mass of 122-143 kDa and a subunit with a molecular mass of 78.5 kDa. Its pH optimum was 8.0 and the \( K_m \) for 4-acetamidobutyrate was 0.29 mM [61].

5.5. Further metabolism of 4-aminobutyrate

From this point, the degradation routes are identical for the acetylated and non-acetylated pathways. In Saccharomyces cerevisiae, Ramos et al. [62] have shown that 4-aminobutyrate aminotransferase and succinate semialdehyde dehydrogenase are induced by growth on 4-aminobutyrate, and that these are the only enzymes involved in its utilization. 4-Aminobutyrate aminotransferase is found at elevated levels in Candida boidinii grown on spermidine and putrescine as well as on 4-acetamidobutyrate and 4-aminobutyrate [63]. Table 3 shows that the levels of all the enzymes of the pathway are indeed elevated in cells grown on spermidine or putrescine or on the intermediates of the N-acetyl pathway.

4-Aminobutyrate aminotransferase has been purified from Candida guilliermondii grown on 4-aminobutyrate as sole N source by Der Garabedian et al. [64]. It does not catalyse the transamination of β-alanine (as confirmed in Table 3): this requires a separate, pyruvate-dependent aminotransferase (EC 2.6.1.18) in most bacteria and yeasts [25].

Only a single succinate semialdehyde dehydrogenase, encoded by the UGA2 gene, is present in Saccharomyces cerevisiae [62]. It is active with both NAD and NADP.
Fig. 4. Subcellular compartmentation of polyamine breakdown in Candida boidinii. Starting material (spermidine) and end-products (malonate semialdehyde and succinate) are italicized and highlighted.
5.6. Occurrence of the N-acetyl pathway in yeasts

Recent unpublished work has examined a series of yeast species for the possible occurrence of the N-acetyl pathway (Table 4). With the exception of acetamidobutyrate deacetylase in one or two yeasts, all the yeasts containing diamine acetyltransferase [54] also contained all of the other enzymes of the N-acetyl pathway. *Candida utilis* will not grow on spermidine and contains little or no polyamine oxidase, but will grow on putrescine and contains most of the other enzymes of the pathway. *Pichia pastoris*, which lacks polyamine oxidase, lacks all the other enzymes of the pathway except acetylputrescine oxidase, which in any case would catalyse the oxidation of free putrescine in this organism. The low levels of succinate semialdehyde dehydrogenase in *Candida boidinii* and *Sporopachydermia cereana* remain to be explained. Non-optimal assay conditions are a possible cause.

5.7. Subcellular compartmentation

The enzymes of polyamine breakdown in yeasts via the N-acetyl route are located in several different subcellular compartments [65]. These subcellular compartments are in most cases not the same as those identified for the corresponding enzymes in mammalian tissues (see [53]). Spermidine, after entering the cell, needs to enter the mitochondria for acetylation (Fig. 4) [54], whence it must move to the peroxisomes for oxidation by polyamine oxidase [46]. The putrescine and 1,3-diaminopropane formed by polyamine oxidase in *Candida boidinii* must return to the mitochondria for further acetylation, thereafter back to the peroxisome for oxidation to 3-acetamidopropion-aldehyde or 4-acetamidobutyraldehyde. The remaining enzymes, aldehyde dehydrogenase, 4-acetamidobutyrate deacetylase and 4-amino-butyrate aminotransferase, are located in the cytosol [60,63]. These intracellular transport processes will make energy demands on the cell. The picture may become more complicated if further work on polyamine accumulation and degradation reveals an involvement of the vacuole in the storage of polyamines or their degradation products, as is known to be the case with various other N sources [66,67].

The studies on yeasts grown on polyamines have not yet produced mutants lacking the ability to utilize spermidine or putrescine as sole N source. If the published suggestions about the role of the acetylated pathway are correct, such mutants should lack one or other of the enzymes of this route.

6. CONCLUSIONS

Although recent developments (e.g. [8]) have improved our understanding of the range of organisms able to catabolize polyamines, our knowledge of the routes between spermidine and putrescine are very fragmentary. The N-acetyl pathway needs to be further confirmed by mutants studies, and it has still to be investigated in bacteria. Overall conclusions are that outside the well characterized bacterial species *Escherichia coli*, our knowledge and understanding of polyamine degradation are extremely incomplete.

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