Polyamine stress at high pH in *Escherichia coli* K-12

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**Abstract**

**Background:** Polyamines such as spermine and spermidine are required for growth of *Escherichia coli*; they interact with nucleic acids, and they bind to ribosomes. Polyamines block porins and decrease membrane permeability, activities that may protect cells in acid. At high concentrations, however, polyamines impair growth. They impair growth more severely at high pH, probably due to their increased uptake as membrane-permeant weak bases. The role of pH is critical in understanding polyamine stress.

**Results:** The effect of polyamines was tested on survival of *Escherichia coli* K-12 W3110 in extreme acid or base (pH conditions outside the growth range). At pH 2, 10 mM spermine increased survival by 2-fold, and putrescine increased survival by 30%. At pH 9.8, however, *E. coli* survival was decreased 100-fold by 10 mM spermine, putrescine, cadaverine, or spermidine. At pH 8.5, spermine decreased the growth rate substantially, whereas little effect was seen at pH 5.5. Spermidine required ten-fold higher concentrations to impair growth. On proteomic 2-D gels, spermine and spermidine caused differential expression of 31 different proteins. During log-phase growth at pH 7.0, 1 mM spermine induced eight proteins, including PykF, GlpK, SerS, DeaD, OmpC and OmpF. Proteins repressed included acetate-inducible enzymes (YfiD, Pta, Lpd) as well as RapA (HepA), and FabB. At pH 8.5, spermine induced additional proteins: TnaA, OmpA, YrdA and NanA (YhcJ) and also repressed 17 proteins. Four of the proteins that spermine induced (GlpK, OmpA, OmpF, TnaA) and five that were repressed (Lpd, Pta, SucB, TpiA, YfID) show similar induction or repression, respectively, in base compared to acid. Most of these base stress proteins were also regulated by spermidine, but only at ten-fold higher concentration (10 mM) at high pH (pH 8.5).

**Conclusion:** Polyamines increase survival in extreme acid, but decrease *E. coli* survival in extreme base. Growth inhibition by spermine and spermidine requires neutral or higher pH. At or above pH 7, spermine and spermidine regulate specific proteins, many of which are known to be regulated by base stress. High pH amplifies polyamine stress; and naturally occurring polyamines may play an important role in base stress.

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**Background**

Polyamines are required for the normal cell growth of *Escherichia coli*, although their functions are poorly understood [1-3]. Polyamines bind nucleic acids and ribosomes, where they are needed for optimal function [4,5]. Excessive intracellular concentrations, however, retard protein synthesis and cell growth [6]. Polyamine metabolism is stimulated by a variety of environmental stresses such as heat shock [7].

The major polyamine of bacteria, putrescine \( \text{[NH}_2\text{(CH}_2\text{)}_4\text{NH}_2 \text{]} \), is synthesized by biosynthetic decarboxylation of arginine and/or ornithine [8,9]. Putrescine is metabolized to spermidine \( \text{[NH}_2\text{(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NH}_2 \text{]} \). Spermine \( \text{[NH}_2\text{(CH}_2\text{)}_3\text{NH(CH}_2\text{)}_4\text{NH(CH}_2\text{)}_4\text{NH}_2 \text{]} \), a longer polyamine commonly produced by eukaryotes, is not produced by *E. coli*. Nevertheless, uptake of exogenous spermine fulfills the bacterial requirement for polyamines [4,5]. Spermine and spermidine do not undergo catabolism by *E. coli*, although excess concentrations are acetylated by polyamine acetyltransferase [10,11].

In the human colon, bacteria excrete putrescine and cadaverine during digestion of high-protein foods. Exposure of colonic epithelium to these polyamines stimulates human cell proliferation and leads to colonic tumors [12], which can be treated by drugs that deplete polyamine content [13]. Thus the modulation of polyamine metabolism under conditions of the gut is an important medical concern.

The inhibition of growth by excess polyamines is amplified at high pH [14]. Polyamine stress enhances translation of the growth phase sigma RpoS [15], whose role in stationary-phase survival involves high pH [16]. A possible explanation for the amplification of polyamine stress at high pH is that polyamines become deprotonated and neutralized, thus capable of crossing the cell membrane as membrane-permeant weak bases. Base-dependent uptake could augment the uptake through transporters [17]. The uptake of amines leads to their accumulation proportional to the transmembrane pH difference (ten-fold for each pH unit). Only a small fraction of an amine needs to be unprotonated (less than 1%) in order for significant membrane passage to occur. The \( pK_a \) values of linear polyamines range from \( pK_a = 8.3 \) to 11.6; for example, Ref [18] reports for 30 mM spermidine values of \( pK_a1 = 8.6 \), \( pK_{a2} = 10.0 \), \( pK_{a3} = 11.1 \). However, literature reports vary for different conditions, and polyamine protonation levels under biological conditions are further influenced by complexing with fatty acids and phospholipids.

At low pH, on the other hand, the transmembrane pH difference (\( \Delta pH \)) limits the entry of exogenous amines; and cytoplasmic production of membrane-permeant amines can enhance bacterial growth. A pH-dependent source of amines in *E. coli* is the degradative arginine and ornithine decarboxylases (AdiA and SpeF respectively) and lysine decarboxylase (CadA), which are induced anaerobically at low pH [19]. The generation of putrescine (by AdiA) or cadaverine (by CadA), followed by excretion via cotransported transporters, neutralizes the acidic external environment [20]; for review, see [21,22].

At low-to-neutral pH, *E. coli* polyamines block the porins OmpF and OmpC, decreasing membrane permeability [23-25]. It was proposed that polyamines contribute to *E. coli* survival in extreme acid, below the growth range [24]; a phenomenon termed acid resistance or acid survival [21,22]. An OmpC mutant in which the porin fails to be blocked by cadaverine shows decreased survival at low pH (acid resistance) in the presence of cadaverine [24]. To our knowledge, it has not been shown directly that exogenous polyamines enhance acid resistance of wild-type cells. If polyamines do enhance survival in extreme acid, they could assist *E. coli* and other enteric pathogens during their passage through the stomach [26].

The interactions between pH and polyamines however remain poorly characterized, and are often discounted in studies of polyamine stress. For example, a recent major study of polyamine-mediated gene regulation does not address pH [3]. We report the effect of exogenous spermine and other polyamines on *E. coli* survival in extreme acid or extreme base. We also present protein profiles of *E. coli* exposed to exogenous polyamines under neutral and alkaline pH conditions, in the context of known pH-dependent expression profiles [27,28].

**Results**

*Survival at extreme pH*

*E. coli* grown at moderate pH values possess mechanisms of protection against more extreme pH; these mechanisms are typically induced during stationary phase, or during growth near the acid or base end of their pH range [21,22]. We tested the effects of spermine, putrescine, spermidine and cadaverine on survival rates in extreme acid or base. We also present protein profiles of *E. coli* exposed to exogenous polyamines under neutral and alkaline pH conditions, in the context of known pH-dependent expression profiles [27,28].

At pH 2.0, the presence of 10 mM spermine doubled the proportion of *E. coli* survivors (Figure 1). Putrescine and
cadaverine increased survival by smaller increments, and spermidine had no effect. At pH 9.8, however, all four polyamines tested decreased survival by more than 100-fold. Thus, the presence of polyamines enhanced survival in acid but drastically diminished survival in extreme base.

Growth rates with spermine or spermidine

In order to observe polyamine stress and protein expression during growth, we treated cultures with exogenous spermine and spermidine. These polyamines were selected because they do not undergo the rapid catabolism that occurs to putrescine, the major endogenous polyamine of *E. coli*. Spermine interacts with cell components in similar ways as other polyamines found in *E. coli* [5], and it effectively enters cells [17].

Growth rates were observed in the presence and absence of spermine at pH 5.5, 7.0, or 8.5 (Figure 2). At pH 7.0 or higher, 3 mM spermine eliminated growth, and at pH 8.5 as little as 1 mM spermine substantially decreased growth. At pH 5.5, however, spermine had no effect up to concentrations as high as 10 mM, and spermidine had no effect at 20 mM (data not shown). Spermidine required larger concentrations to affect growth at high pH. At pH 7.0, spermidine concentrations up to 15 mM did not affect the generation time, but at pH 8.5, 15 mM spermidine prevented growth. Thus both spermine and spermidine showed base-dependent depression of *E. coli* growth, although spermidine required higher concentrations.

2-D protein gels

The protein profiles of *E. coli* in the presence and absence of exogenous spermine or spermidine were observed at pH 7.0 and at pH 8.5, using methods that previously revealed pH-dependent protein profiles [28,29]. The concentrations of each polyamine were chosen based on Figure 2 so as to cause significant stress at high pH without preventing growth.

The composite 2-D protein profiles are shown in Figures 3 and 4, and the results of quantitative analysis of pairwise comparisons are shown in Table 1. At pH 7.0, 1 mM spermine increased the expression of eight proteins, including PykF, GlpK, SerS, DeaD, OmpC and OmpF. On the other hand, 19 proteins were repressed, including acetate-inducible proteins (YfiD, Pta, Lpd), the RNA polymerase-binding protein RapA (HepA), and the fatty acid synthesis proteins FabB, FabE. At pH 8.5, spermine induced all the proteins induced at pH 7.0, plus four additional proteins: TnaA, OmpA, NanA, and YrdA. Spermidine regulated...
most of the same proteins as spermine, although 10-fold higher concentration was required.

The proteins induced by spermine and spermidine include known base-inducible proteins such as TnaA, which deaminates several amino acids [29,30], as well as base-inducible GlpK, OmpA, and OmpF [27]. Proteins inducible at low pH or by acetate, however, were repressed by spermine, including Lpd, Pta, SucB, TpiA, and YfiD [27,31]. By contrast, at pH 5.5, spermine and spermidine had no effect on protein profiles (data not shown). The lack of effect of polyamines at low pH is consistent with their exclusion from the cell as permeant bases, in equilibrium with the trans-membrane ∆pH.

Several porins (OmpC, OmpF, and OmpA) showed increased expression in the presence of spermine. While blockage of porin function by polyamines is eliminated at pH 9.5 [32], some porin blockage may occur as high as pH 8.5. A possible interpretation could be that cells respond to porin blockage by overexpressing the porins.

The polyamines consistently repressed RapA, a general transcriptional activator that stimulates recycling of RNA
Table 1: Proteins showing differential expression on 2-D gels.

| Spot no. | Protein | Spermine (1 mM) pH 7.0 | Spermine (1 mM) pH 8.5 | Spermidine (10 mM) pH 8.5 | Known or predicted function |
|----------|---------|-------------------------|-------------------------|---------------------------|-----------------------------|
| 1        | PykF    | 0.34 ± 0.09 (+)         | 0.39 ± 0.06 (+)         | Pyruvate kinase            |
| 2        | PykF    | 0.39 ± 0.06 (+)         | 0.39 ± 0.09 (+)         | Pyruvate kinase            |
| 3        | GlpK    | 0.68 ± 0.09 0.63 ± 0.12 | 0.50 ± 0.09             | Glycerol kinase            |
| 4        | SerS    | 0.83 ± 0.08 0.60 ± 0.1  | 0.60 ± 0.1              | Serine-tRNA ligase          |
| 5        | ThrC    | -0.34 ± 0.06           | 0.51 ± 0.03 0.81 ± 0.07 | Threonine synthase         |
| 6        | TnaA    | 0.92 ± 0.06 0.87 ± 0.09 | 0.77 ± 0.07             | Tryptophanase              |
| 7        | MalE    | -1.04 ± 0.05           | -0.74 ± 0.08            | Maltose-binding, periplasmic |
| 8        | MalE    | -1.0 ± 0.00            | -0.54 ± 0.10            | Maltose-binding, periplasmic |
| 9        | MalE    | -0.74 ± 0.08           |                      | Maltose-binding, periplasmic |
| 10       | Asd     | -0.54 ± 0.10           | -0.42 ± 0.07            | Aspartate semialdehyde dehydrogenase |
| 11       | FabB    | -0.47 ± 0.06 (-)       |                      | Galactose-binding protein  |
| 12       | OmpC    | 0.27 ± 0.03 0.41 ± 0.08 | 0.56 ± 0.05 0.74 ± 0.15 | Outer membrane protein C   |
| 13       | TktA    | -0.46 ± 0.11 (-)       |                      | Transketolase              |
| 14       | OmpF    | 0.96 ± 0.03 0.74 ± 0.15 |                      | Outer membrane protein porin |
| 15       | OmpF    | 0.56 ± 0.05 0.90 ± 0.08 |                      | Outer membrane protein porin |
| 16       | Pta     | -0.47 ± 0.06 (-)       |                      | Phosphate acetyltransferase |
| 17       | Pta     | 0.26 ± 0.04 0.23 ± 0.02 |                      | Galactose-binding protein  |
| 18       | MalM    | 0.69 ± 0.11 (-)        |                      | Maltose periplasmic protein |
| 19       | SucB    | -0.25 ± 0.02 -0.89 ± 0.08 |                      | Dihydrolipoamide succinyltransferase |
| 20       | GlnS    | -0.59 ± 0.11 -0.60 ± 0.1 |                      | Glutaminyl-tRNA synthetase  |
| 21       | Lpd     | -0.79 ± 0.12 -0.69 ± 0.1 |                      | Dihydrolipoamide dehydrogenase |
| 22       | Lpd     | -0.55 ± 0.1 -0.72 ± 0.17 |                      | Dihydrolipoamide dehydrogenase |
| 23       | Lpd     | -0.58 ± 0.1 -0.27 ± 0.05 |                      | Dihydrolipoamide dehydrogenase |
| 24       | Lpd     | -0.24 ± 0.04 -0.82 ± 0.07 |                      | Acetyl-CoA carboxylase      |
| 25       | Lpd     | -0.26 ± 0.05 (-)       |                      | 30S ribosomal subunit protein S2 |
| 26       | Lpd     | 0.26 ± 0.04 0.44 ± 0.12 |                      | 30S ribosomal subunit protein A |
| 27       | Lpd     | 0.27 ± 0.04 0.43 ± 0.09 |                      | Outer membrane protein A    |
| 28       | Lpd     | 0.22 ± 0.01 -0.85 ± 0.09 |                      | 30S ribosomal subunit protein S6 |
| 29       | Lpd     | 0.22 ± 0.01 -0.85 ± 0.09 |                      | 30S ribosomal subunit protein S6 |
| 30       | Lpd     | 0.18 ± 0.03 0.40 ± 0.08 |                      | Triosephosphate isomerase   |
| 31       | Lpd     | -0.28 ± 0.03 -0.57 ± 0.06 |                      | Triosephosphate isomerase   |
| 32       | Lpd     | -0.61 ± 0.02 -0.76 ± 0.09 |                      | RNA polymerase binding protein |
| 33       | Lpd     | -0.74 ± 0.02 (-)       |                      | Pyruvate formate-lyase homolog |
| 34       | Lpd     | -0.75 ± 0.1 (-)        |                      | Inosine-5’-monophosphate dehydrogenase |

1Relative differential expression of protein, shown as LDE ± standard error (n = 9), was determined as described under Materials and Methods. Symbols: (+), induced; (-) repressed, representing spots that showed differential expression based on visual inspection, although not quantifiable by the Z3 software.
polymerase [33]; its expression is growth phase depend-
ent, peaking in early log phase [34]. The repression of
RapA could contribute to the growth inhibition by
polyamines at high pH.

Discussion
We show that the presence of spermine enhances bacterial
survival in extreme acid, as well as diminishing survival in
extreme base. This finding is of medical significance, as it
suggests that exogenous polyamines enhance survival
during passage through the stomach to the colon. The lumen
of the colon, at pH 6–8, normally contains millimolar
concentrations of polyamines [12], and levels may reach
5–10 mM in children with diseases of nutrient malabsorp-
tion such as cystic fibrosis [35]. Polyamines are implicated
in colonic hyperproliferation and cell migration, leading
to tumorigenesis.

The polyamine enhancement of extreme-acid survival
could involve several possible mechanisms. Amines pro-
duced by amino-acid decarboxylases can neutralize acid-
ity, a factor in acid resistance [19]. The OmpC porin is
blocked by the polyamine cadaverine at low to neutral
pH; the blockage of porins could protect cells from acidifi-
cation [23-25]. Polyamines could also protect the cell’s
DNA and RNA from effects of internal acidification during
extreme-acid exposure [36].

In protein profiles, spermine and spermidine showed
base-dependent regulation of 31 proteins, including nine
proteins known to respond to base stress. Polyamines
have numerous effects upon cell function; their effects
could be enhanced at high pH by ΔpH-driven uptake of
polyamines. Alternatively, polyamines could amplify base
stress by taking up protons within the cytoplasm, thus
requiring the cell to spend more energy maintaining pH
homeostasis.

Our results of protein expression differ significantly from
the report of putrescine-dependent genes by Yoshida and
colleagues [3]. For example, Ref. [3] reports putrescine
induction of acid stress genes such as dps, hdeA, hdeB, gat-
ABD, and the flagellar regulon. We however find mainly
induction of base stress proteins and repression of acid
stress proteins. The difference may relate to the fact that in
Ref. [3], the growth medium used was glucose-minimal
Medium A, with pH unspecified but presumably buffered
with phosphate at pH 7, with cells grown to late log phase.
These growth conditions involve substantial acidification
and acetate production. Furthermore, the putrescine
microarray study was performed using a mutant deficient
for putrescine conversion to spermidine, which requires
exogenous polyamines for optimal growth. The effects of
acidification could have been amplified by the growth
defect of the polyamine-deficient strain; thus, culture
acidification could explain why Ref. [3] found acid stress
induction. Our proteomic experiments, however, used an
E. coli K-12 strain with no known polyamine defects, and
growth pH was maintained by buffering at pH 7.0 or 8.5.

Conclusion
It has been shown that polyamines increase survival in
extreme acid, but decrease E. coli survival in extreme base.
At or above pH 7, spermine and spermidine induce spe-
cific proteins, including those associated with base stress.
Base stress and base-enhanced polyamine uptake should
be considered during all studies of polyamine stress.

Methods
Growth conditions
E. coli K-12 strain W3110 was grown overnight in potas-
siu-modified Luria broth (LBK) (10 g/l of tryptone, 5 g/
l of yeast extract, 7.45 g/l of KCl). The overnight cultures
were diluted 500-fold into 20 ml of buffered medium
supplemented with 1 mM spermine or 10 mM spermi-
dine. The buffers used included 3-(N- morpholino)pro-
panesulfonic acid (MOPS) (pK a 7.01), 3- [N-
tris(hydroxymethyl)methyl]- 3-aminopropanesulfonic
acid (CAPS) (pK a 10.4). Buffers and polyamines were purchased from Research Organics and
from Sigma. The pH values of media were adjusted by
using KOH to avoid extra sodium ions, which stress cells
at high pH [37]. The pH was tested after culture growth to
ensure that the values were maintained at ± 0.1 pH unit of
the pH of the original uninoculated medium. For 2-D
gels, all cultures were grown aerobically in baffled flasks
rotated at 240 cycles/sec at 37°C until the OD 600 reached
0.2.

Survival assays
To assay survival in extreme acid, overnight cultures of
bacteria in LBK 50 mM MOPS pH 7.0 were diluted 1000-
fold in LBK adjusted to pH 2.0. After 2 h incubation at
37°C, the pH of the culture was tested to confirm mainte-
nance at pH 2.0. Cells were plated on LBK agar at 37°C.
For survival in extreme base, overnight cultures in LBK 50
mM TAPS pH 8.5 were diluted 1000-fold in LBK 100 mM
CAPS pH 9.8, incubated for 2 h at 37°C, and the pH tested
to confirm maintenance at pH 9.8. Dilutions were plated
on LBK. For either acid or base experiments, survival was
normalized to that of control samples diluted in LBK 50
mM MOPS pH 7.0 and plated directly without
incubation.

2-D protein gel electrophoresis
2-D gel electrophoresis of proteins was performed as
described [28,31,38]. The protein mixtures were first sep-
parated by isoelectric focusing using 18-cm polyacrylamide
gel strips with an immobilized pH 4 to 7 gradient (AP Bio-
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