POLYAMINE METABOLISM IN AN OBLIGATELY ALKALOPHILIC

BACILLUS ALCALOPHILUS THAT GROWS AT PH 11.0

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Summary: Bacillus alcalophilus, an obligately alkalophilic bacterium that grows at pH 11.0, has an intracellular pH of 9.5 or less. Unlike all other living organisms, polyamines (putrescine, spermidine and spermine) in B. alcalophilus, if present, will be largely unprotonated. HPLC analysis indicated that spermidine is the major polyamine in B. alcalophilus, accounting for more than 90% of total polyamines, and the level of spermidine varies during growth. Ornithine decarboxylase activity was not detectable in B. alcalophilus under all conditions examined. When [^{3}H]arginine was added to the culture medium, the radioactivity can be recovered from polyamine pool; the distribution is 3% for putrescine, 94% for spermidine, and 3% for spermine, suggesting the presence of arginine pathway for polyamine biosynthesis. The polyamine transport system in B. alcalphilus appears to be Na$^+$-dependent and is highly sensitive to the inhibition of gramicidin S and valinomycin. © 1988 Academic Press, Inc.

Polyamines (putrescine, spermidine, and spermine) are naturally occurring

organic cations widely distributed in living organisms [1,2]. Abundant

evidence in literature indicates that polyamines are essential for growth in

both prokaryotes and eukaryotes [3-5]. Many metabolic pathways are involved

in modulating polyamine contents in cells [3-5]. Polyamines interact with

proteins and nucleic acids and have been shown to affect a wide spectrum of

biological reactions in vitro [3-6]. Because of the abundance and versatility

of polyamines in interacting with other biological molecules, it has been

difficult to define precisely the function of individual polyamines in vivo.

Recently, attention has been directed to the study of polyamine metabolism in

unusual organisms such as extreme halophilic bacteria [7,8] and thermophilic

bacteria [9,10] in the hope that comparative studies may shed more light on

the function and physiological significance of polyamines.

Bacillus alcalophilus belongs to a group of bacteria that have an optimal pH of growth at 10.5 or above, and a cytoplasmic pH of 9.5 or below [11]. Values of the dissociation constants (pKa) for various polyamines are in the ranges of 8.5 to 10 [12,13]. Thus, for spermidine, each nitrogen is fully protonated at pH 7.0 but only ~30% will be protonated at pH 10.5 [13]. In view of this, polyamines in B. alcalophilus, if present, will be much less polybasic than that in other organisms. If the polybasic property of polyamines is a key to their physiological function, differences in polyamine metabolism between extreme alkalophiles and other organisms may be expected.

In this paper, we have determined the polyamine contents and examined the biosynthetic pathways of polyamines in B. alcalophilus. In view of the "reversed" proton gradient [11,15] in this organism, we also studied its polyamine transport system.

MATERIAL AND METHODS

Bacterial Strains. A culture of Bacillus alcalophilus was a gift of Dr. T.A. Krulwich, Mt. Sinai Medical School, NY. Escherichia coli (ATCC 25922) was previously obtained from Difco Lab, Detroit, MI.

Growth and Harvesting. The basal growth medium for B. alcalophilus consisted of 25mM Na_2CO_3 buffer, 1mM KH_2PO_4/K_2HPO_4, 0.1% (w/v) $(NH_4)_2SO_4$, and 0.1 mM $MgSO_4 \cdot 7H_2O$ in H_2O; KOH was used to obtain the final pH of 11.0 prior to the addition of $MgSO_4$. This basal medium was supplemented with 0.1% yeast extract, 1% (v/v) trace metal solution [14], and 50 mM sodium L-malate added from separate sterile solutions [15]. Samples of 5 to 10 ml were harvested by centrifugation at 27,000xg for 10 min; samples of less than 1.5 ml were centrifuged at 12,000 rpm for 3 min using Beckman Microfuge. Cell pellets were washed twice with basal growth medium. E. coli was grown in BBL Trypticase Soy Broth. Cells were harvested, washed and suspended in phosphate buffered saline unless indicated otherwise.

Identification and Quantitation of Polyamines. The procedure of Seiler and Weichman [16] was used to dansylate polyamines extracted from B. alcalophilus and E. coli. The dansylated polyamines in cell extracts were separated by HPLC on a reverse-phase columnm and quantitated by a Schoeffel spectro-fluorimeter as we previously described [7,17]. For metabolic conversion experiments, dansylated polyamines were separated on Whatman K5 silica gel TLC plates in $CHCl_3$: $(C_2H_5)_3N$ (5:1 v/v) [18]. Spots corresponding to standard polyamines were scraped from the plate for radioactive counting.

Assay for Ornithine Decarboxylase (ODC) Activity. Aliquots of 5 ml E. coli or 10 ml B. alcalophilus cells were removed from cultures at various times after a 1:10 dilution with fresh growth medium, centrifuged, washed, and resuspended in a Tris buffer (50 mM Tris, pH 7.4 or 9.5) containing 50 μM pyridoxal-5'-phosphate, 0.1 mM EDTA, and 5 mM dithiothreitol (TEPD buffer). The cells were

sonicated and centrifuged at 12,000 rpm for 5 min. The supernatant was used for assay of ODC activity by a procedure previously described [17].

Putrescine Uptake.   B. alcalophilus cells were washed with basal growth medium and resuspended in the same medium containing [³H]putrescine and various drugs. At designated time points, 1.5 ml of cell suspension was removed, filtered (Millipore 1225 Sampling Manifold) through Millipore nitro-cellulose filters, and washed. The filters were air dried and counted in Aqualyte by a liquid scintillation spectrometer.

RESULTS AND DISCUSSION

As an initial step to investigate polyamine metabolism in B. alcalophilus, we examined the levels of individual polyamine (putrescine, spermidine and spermine) in this organism during its growth at pH 11.0. Results obtained from two separate experiments were shown in Fig. 1. Spermidine was the major polyamine in B. alcalophilus, accounting for more than 90% of total polamines. In contrast, 80% of total polyamines in E. coli is putrescine [19]. Spermidine level in B. alcalophilus increased and reached a peak level 1~2 hrs after the initiation of growth. Levels of putrescine and/or spermine, however, remained either low or barely detectable throughout the entire time course of growth (Fig. 1). Since most prokaryotic cells contain only putrescine and spermidine [1-3], the presence of minute quantity of spermine in B. alcalophilus could be due to contamination from yeast extracts in the supplemented growth medium. To test this possibility, bacterial cultures were
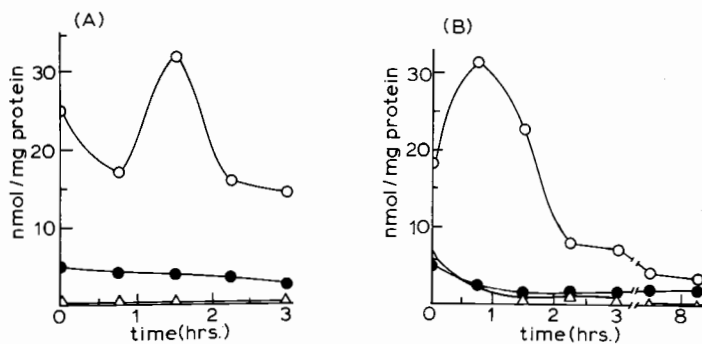

Fig.1.   Changes of polyamine contents in B. alcalophilus during growth.
Overnight cultures were diluted 10-fold with fresh supplemented growth medium for (A) 1.5 hrs or (B) 3 hrs and further diluted 5-fold with fresh supplemented growth medium. Polyamine contents in two cultures were then determined at various times after the second dilution as described under MATERIAL and METHODS. Putrescine ($\triangle$), spermidine ($\bigcirc$). spermine ($\bullet$).

incubated with [^{3}H]arginine for various times and the distribution of
radioactivity in polyamine pool was analyzed by TLC and liquid scintillation
spectrometry. For a comparison, we also carried out a similar metabolic
conversion experiment with E. coli. Radioactivity in the polyamine pool of B.
alcalophilus appeared in putrescine, spermidine, and spermine with more than
90% of this radioactivity recovered as spermidine (Table 1). In contrast,
more than 95% of the radioactivity in the polyamine pool of E. coli appeared
as putrescine, and no radioactivity was detectable in the spot corresponding
to spermine (Table 1). Thus, our data suggest the presence of biosynthetic
pathway for spermine in B. alcalophilus but not in E. coli.

In prokaryotes, two synthetic pathways are known to be involved in
putresine production, one uses arginine and the other uses ornithine as
precursor [20]. The key enzyme for the ornithine pathway is ornithine
decarboxylase (ODC, EC 4.1.1.17). Specific ODC activity was induced in E.coli
during growth but was not detected in B. alcalophilus throughout the entire
period of growth, whether the assay was carried out at pH 7.2 or 9.5 (Fig. 2).

TABLE 1

Metabolic Conversion of Arginine to Polyamines in B. alcalophilus

Sample	Putrescine	Spermidine	Spermine
	(Counts per minute per spot)		
B. alcalophilus			
3-hour	7±2[a]	232±94	8±2
6-hour	10±6	202±108	8±7
E. coli			
3-hour	416±252	12±12	ND[b]
6-hour	477±12	10±2	ND

[a]The number is the difference of counts between dansylated polyamine
spots and backgrounds. Each number represents an average of two
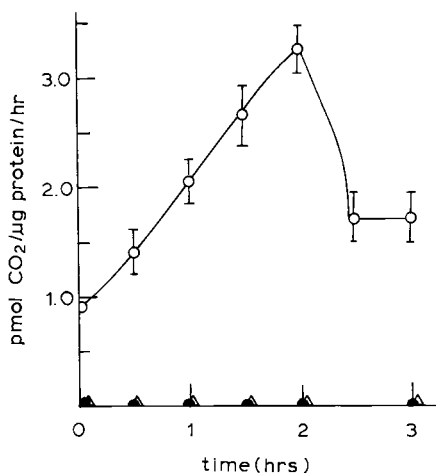separate experiments.

[b]ND, nondetectable.

Fig.2.   Ornithine decarboxylase activity in E. coli (O) and B. alcalophilus
(● , Δ). Overnight cultures of E. coli and B. alcalophilus were
diluted 10-fold with fresh supplemented growth medium. Aliquotes of
cells were removed at various times after dilution for ODC assay. For
B. alcalophilus, ODC activity was measured at both pH 7.2   (●) and
pH 9.5 (Δ).

In accordance with this observation, no appreciable metabolic conversion was

observed in B. alcalophilus when [3H]ornithine was added to the growth medium

(data not shown). Together, these results suggest that, unlike most other

prokaryotes, the ornithine pathway in B. alcalophilus is either missing or

not operational.

An additional source of polyamines in living organisms comes from the

transport of polyamines from growth medium [3,20]. Active transport system

for polyamines have been described for E. coli [19] and cultured mammalian

cells [21]. Because of the low proton motive force in B. alcalophilus [11],

it is generally thought that this organism may possess unusual transport

systems for amino acids and other nutrients. Studies carried out by Krulwich

and associates have demonstrated that obligately alkalophilic bacteria possess

Na+/solute symports rather than H+/solute symports that are typically found in

other bacteria [11,15]. In light of their work, and considering the chemical

nature of polyamines at pH 11, it is of interest to examine the polyamine

transport system in B. alcalophilus. We found that putrescine uptake in B.

alcalophilus followed a Michaelis-Menten kinetics with apparent Km and Vmax

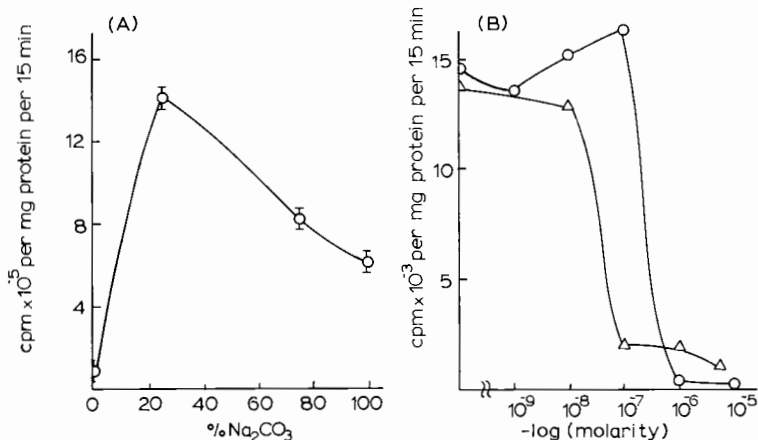values of 3.3 μM and 31 nmol/mg protein/hr, respectively, (data not shown),

Fig.3.   The sodium dependency and the effect of ionophores on putrescine
transport in $\underline{B}$. alcalophilus.   (A) The uptake of [^{3}H]putrescine (0.5
μCi/ml, 5 μM) in $\underline{B}$. alcalophilus was measured for 15 min in the basal
growth medium where Na_2CO_3 was displaced by K_2CO_3.   At the end of
incubation at 36°C, cells were washed and cell-associated
radioactivity was determined as described under MATERIAL and METHODS.
Each point represents an average of duplicate measurements.   (B) The
uptake of [^{3}H]putrescine (10 nCi/ml, 5 μM) in $\underline{B}$. alcalophilus was
measured for 15 min in the basal growth medium containing gramicidin S
(-0-) or valinomycin ($-\triangle-$) at various concentrations.

suggesting the presence of a carrier mediated transport system.   This

transport system exhibited a biphasic Na^+-dependency with maximal uptake

occurring at 30% Na_2CO_3 (Fig. 3A).   Ionophores such as gramicidin S and

valinomycin, known to disrupt the normally existing transmembrane Na^+/K^+

balance, were found to be potent inhibitors of putrescine uptake (Fig. 3B).

Thus, the polyamine transport in $\underline{B}$. alcalophilus appears to depend on an

electrochemical gradient, possibly via a Na^+/putrescine symport mechanism.

Polyamine metabolism in extremely alkalophilic bacteria has not been

previously investigated.   Our results indicated that spermidine is the major

polyamine in $\underline{B}$. alcalophilus and that its biosynthesis depends solely on

arginine pathway.   We also showed that putrescine uptake in this organism

involves a carrier system and an electrochemical gradient.   These data should

serve as a basis for further characterization of the regulation of polyamine

metabolism and functions of polyamines in this unusual organism.

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