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Polyamines of *Pseudomonas acidovorans*

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Pseudomonas acidovorans contains putrescine, 2-hydroxyputrescine, and spermidine.

Almost one-half of the thymine residues in bacteriophage ϕW-14 deoxyribonucleic acid are replaced with *N*-thyminylputrescine (6). As a preliminary to investigating the biosynthesis of this unusual pyrimidine, the polyamines present in *Pseudomonas acidovorans*, the host organism, have been identified.

P. acidovorans strain 29 (see reference 7) was grown in liquid minimal salts medium (1), without NaCl and with 4 g of disodium succinate per liter. Cells were harvested and washed by centrifugation at room temperature to minimize possible nonphysiological acetylation of polyamines (11). Polyamines were extracted from the washed cells by the method of Raina (9).

Thin-layer chromatography showed that *P. acidovorans* contained three polyamines (Table 1), two of which, by comparison with authentic standards, were identified as spermidine and putrescine. The R_f of the third compound in solvent C indicated that it might be 2-hydroxyputrescine (13) or 1,3-diaminopropane.

The unknown polyamine was purified from log-phase cells of *P. acidovorans* (10-liter fermentor culture harvested at an optical density of 1.0 at 650 nm with a Sharples centrifuge) by butanol extraction (9) and cation exchange chromatography on Dowex 50H$^+$-X2, 100 to 200 mesh (12). The unknown compound was eluted from the column just before putrescine (see reference 5). The final yield of purified product, which was chromatographically homogeneous, was 22 mg. It gave a purple color with ninhydrin and a positive reaction with periodate. The nuclear magnetic resonance spectrum (in D_2O, relative to an external tetramethylsilane signal at δ = 0), which showed 2.25 (2H, —CH_2—), 3.49 (4H, —CH_2N—) and 4.37 (1H, —CH—O—), confirmed that the compound was 2-hydroxyputrescine (see reference 13).

The intracellular concentrations of the polyamines were determined fluorometrically after dansylation of extracts (2) by using a Turner model 111 fluorometer fitted with a thin-layer scanner. Assuming that *P. acidovorans* has approximately the same cell volume as *Escherichia coli*, mid-log-phase cells contained about 50 mM putrescine, 3 to 5 mM spermidine, and 45 mM hydroxyputrescine.

TABLE 1. *Thin-layer chromatography of polyamines*[a]

Compound	R_f in solvent		
	A	B	C
Spermidine	0.24	0.25	0.10
Putrescine	0.40	0.38	0.22
Pseudomonas acidovorans extract	0.20, 0.32, 0.39	0.29, 0.39	0.11, 0.24 0.38

[a] Solvent systems: A, diethylene glycolmonoethyl ether-propionic acid-water (70:15:15) saturated with NaCl (reference 2); B, isopropanol-concentrated HCl-water (80:30:20) (reference 3); and C, methanol-concentrated NH_4OH (reference 9). Eastman cellulose sheets were used with solvents A and B, and Eastman silica gel sheets were used with solvent C. Authentic spermidine and putrescine were obtained from Calbiochem.

To our knowledge, *P. acidovorans* is the first bacterium reported to contain both spermidine and 2-hydroxyputrescine. *Pseudomonas* (Kim) contains 2-hydroxyputrescine, but not spermidine (5, 8, 13), and, because both compounds have three hydrogen-bonding sites, it was suggested that 2-hydroxyputrescine may not occur in cells containing spermidine (8, 10). It is possible that 2-hydroxyputrescine can assume the roles played by spermidine, but the occurrence of both compounds in *P. acidovorans* suggests that, in this organism at least, they may play distinct roles.

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