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Polyamine Distribution Patterns in *Pseudomonas*, *Alcaligenes* and *Comamonas*

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SUMMARY: *Pseudomonas cepacia* as well as all five *Alcaligenes* and three *Comamonas* species tested belonging to the beta subclass of *Proteobacteria* ubiquitously contained 2-hydroxyputrescine and putrescine as the major polyamines. Cadaverine was found in *P. cepacia*. Putrescine and spermidine were the major polyamines of *Pseudomonas aeruginosa* and *Pseudomonas putida* belonging to the gamma subclass and *Pseudomonas saccharophila* whose taxonomic positions have not been established. Polyamine distribution profiles can serve as a chemotaxonomic marker within *Pseudomonas* species. Relative distribution patterns of the major polyamines of *P. aeruginosa* did not changed at different culture periods in spite of increase or decrease of their levels during growth.

INTRODUCTION

Polyamines have been shown to serve as chemotaxonomic markers within *Proteobacteria* which include the majority of the well-known Gram-negative eubacteria (1). Nearly identical polyamine distribution patterns are found in the species belonging to the same subclasses in *Proteobacteria* (2-6). The alpha subclass contains putrescine and homospermidine, the beta subclass 2-hydroxyputrescine and putrescine and the gamma subclass putrescine and spermidine, as the main polyamine components.

Pseudomonas species are located on three rRNA branches; rRNA superfamilies I+II, III and IV corresponding to the gamma, beta and alpha subclass of *Proteobacteria*, respectively (1, 7). In the present study, polyamines of some *Pseudomonas* species belonging to the beta or gamma subclass and various species of the genus *Alcaligenes* and *Comamonas* belonging to the beta subclass were analyzed to validate their taxonomic position on the basis of polyamine distribution patterns.

To establish polyamine profiles as a chemotaxonomic marker, constancy of the patterns during growth should be demonstrated. Changes in cellular polyamine levels at different growth periods were determined in *P. aeruginosa*.

MATERIALS AND METHODS

The organisms were cultured at 30℃ in synthetic 199 Medium (polyamine-free) (Flow Lab. Irvine, UK). The organisms were harvested after shaking culture for 34 hr (Table 1

) or at four culture periods (Table 2). The pellets were homogenized (for Table 1) or sonicated (for Table 2) in 0.5 M perchloric acid ($HClO_4$). Polyamines were extracted into $HClO_4$ and analyzed by high-performance liquid chromatography (HPLC) on a column (4 mm ID x 50 mm) of cation-exchange resin (Hitachi #2619F) at 70℃. The buffers used were 0.045 M sodium citrate-0.060 M citric acid and 0.20 M sodium citrate containing 0.064 M and 2.0 M NaCl, respectively. The elution patterns were followed by *o*-phthalaldehyde. Before HPLC analysis, acid-hydrolysis, alkaline-hydrolysis and periodate oxidation of the polyamine samples were also performed to confirm the identity of polyamine peaks (8, 9). Sonicated bacterial samples were subjected to the protein assay by Lowry method (10).

RESULTS AND DISCUSSION

Polyamine profiles in *Pseudomonas*, *Alcaligenes* and *Comamonas*

Typical chromatograms of the HPLC analysis of *Pseudomonas*, *Alcaligenes* and *Comamonas* species belonging to the beta subclass of *Proteobacteria* are shown in Fig. 1. Polyamine contents expressed as μ mol per wet weight in four *Pseudomonas* species, five *Alcaligenes* species and three *Comamonas* species are listed in Table 1.

Putrescine plus homospermidine-type (the first polyamine type) has been found in some *Pseudomonas* belonging to the alpha subclass such as *P. diminuta*, *P. aminovorans*, "*P. azotocolligans*", "*P. compransoris*", "*P. carboxydovorans*" and *P. carboxydohydrogena* (2, 4, 11). However, this polyamine distribution type was not found in the organisms tested in the present study (Table 1).

P. cepacia as shown in Fig. 1 and Table 1, as well as *P. solonacearum*, *P. caryophylli*, *P. marginata* and "*P. thermocarboxydovorans*" which have the second polyamine type (2-hydroxyputrescine and putrescine (and spermidine)) (4, 11), belong to the beta subclass. Hydrogen pseudomonads, "*P. hydrogenothermophila*" and "*P. hydrogenovora*" also contained 2-hydroxyputrescine in addition to putrescine and spermidine (8). Four hydrogen-oxidizing *Hydrogenophaga*, *H. flava*, *H. palleronii*, *H. pseudoflava* and *H. taeniospiralis*, formerly classified in *Pdeudomonas*, belonging to the beta subclass, have this polyamine type (12). *P. cepacia* contained cadaverine in addition to 2-hydroxyputrescine and putrescine as a unique polyamine pattern in the *Proteobacteria* belonging to the beta subclass (Fig. 1 and Table 1). H_2-oxidizing, N_2-fixing *P. saccharophila* placed in the beta subclass but diverged from H_2-oxidizing *Hydrogenophaga* (12, 13) lacked 2-hydroxyputrescine, as shown in Table 1. Thus, *P. saccharophila* would not belong to the beta subclass, though it may be insufficient to draw a final conclusion as to the taxonomic position of this organism.

Authentic *Pseudomonas* species such as *P. aeruginosa* and *P. putida* placed in the gamma subclass contained putrescine, cadaverine and spermidine (the third polyamine type), as shown in Fig. 1 and Table 1. Other pseudomonads belonging to the gamma subclass, *P. alcaligenes*, *P. chlororaphis*, *P. fluorescens* and *P. mendocina* contained also the same polyamine components (4, 5).

Our polyamine analysis of various strains of *Alcaligenes* and *Comamonas* comfirmed here the results reported by Busse and Auling (4). *Alcaligenes* including *Variovorax paradoxus* (formerly *A. paradoxus*) (14, 15) and *Comamonas* in which some of them formerly placed in *Pseudomonas* (16), which are now classified to be the beta subclass of

Proteobacteria showed the second type of polyamine pattern (Fig. 1 and Table 1). 2-Hydroxyspermidine, a novel polyamine, was found in *C. acidovorans* (IFO 13582=ATCC 9355) as detected in *P. acidovorans* 29 (17), "*P. thermocarboxydovorans*" (11) and "*P. hydrogenothermophila*" (8). Further grouping and separation are expected for the beta subclass which consists of Comamonadaceae (*Comamonas, Xylophilus, Hydrogenophaga, Variovorax, Acidovorax, Aquaspirillum*) (15) and Alcaligeneceae (14) on the basis of polyamine profiles.

Cellular polyamine concentrations in *Pseudomonas aeruginosa*

Polyamines of several PAO1 strains of *P. aeruginosa* maintained in two different institutions, Gunma University and Tokai University, were analyzed. Almost identical polyamine levels (nmol per mg protein) were detected among these strains, as shown in Table 2. Some fluctuations in cadaverine and spermidine levels were detected during culture (Fig. 2 and Table 2). Histamine was found in the cultures at 48 hr. However, distribution profiles of major polyamines are nearly the same in various strains of *P. aeruginousa* PAO1 harvested at different growth periods. These findings support the assumption that polyamine distribution patterns can serve as a phenotypic marker relevant to chemotaxonomy within heterogeneous *Pseudomonas* species.

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Table 1. Cellular concentrations of polyamines in *Pseudomonas*, *Alcaligenes* and *Comamonas*.

	Polyamine (μmol/g wet cell)				
	H-Put	Put	H-Spd	Cad	Spd
P. cepacia GUNMA	0.64	3.50	ND	1.32	0.02
P. aeruginosa PAO1 GUNMA(Iyobe)	ND	4.00	ND	1.50	2.00
P. putida TN 112 GUNMA	ND	4.20	ND	0.04	3.10
P. saccharophila IAM 1504 (=ATCC 15946)	ND	0.90	ND	0.24	0.72
A. eutrophus					
IAM 12627 (=ATCC 33178) ("*A. hydrogenophilus*")	0.80	2.20	ND	0.01	ND
IAM 13533	2.40	3.50	ND	ND	ND
IAM 13548	0.31	0.56	ND	ND	0.35
IAM 13549	0.65	0.65	ND	0.01	0.04
A. latus					
IAM 12599 (=ATCC 29712)	0.70	0.95	ND	0.02	0.10
IAM 12664	0.35	0.45	ND	ND	0.18
IAM 12665	0.24	0.55	ND	ND	ND
A. paradoxus					
IAM 13535	0.10	1.07	ND	ND	ND
IAM 12373	0.20	0.40	ND	ND	ND
IAM 12374	1.15	1.35	ND	ND	0.04
IAM 13551	0.30	1.30	ND	ND	0.07
A. denitrificans					
IAM 12600 (=ATCC 15749) (*A. ruhlandii*)	1.87	0.50	ND	ND	ND
IAM 12370 (=ATCC 15173)	3.00	2.25	ND	ND	0.10
IAM 12556	1.20	1.85	ND	ND	ND
IAM 12563	2.60	3.00	ND	ND	ND
JCM 5490 (=ATCC 15173)	2.30	2.50	ND	ND	ND
A. faecalis					
JCM 1474 (=ATCC 8750)	3.00	3.00	ND	ND	ND
JCM 5485 (=ATCC 15554) ("*P. odorans*"="*A. odorans*")	0.15	2.50	ND	ND	0.60
IAM 12369	0.24	2.50	ND	ND	0.80
IAM 12560	0.32	3.00	ND	ND	0.52
IAM 12561	0.62	1.72	ND	ND	0.30
IAM 12372	0.30	1.60	ND	ND	0.27
C. acidovorans					
IFO 13582 (=ATCC 9355) (*P. acidovorans*)	0.79	1.18	0.02	ND	0.30
C. terrigena					
IFO 13299 (=ATCC 8461)	0.81	0.35	ND	ND	ND
C. testosteroni					
IAM 12419 (*P. testosteroni*)	0.32	0.95	ND	ND	0.02

Abbreviations: H-Put, 2-hydroxyputrescine; Put, putrescine; H-Spd, 2-hydroxyspermidine; Cad, cadaverine; Spd, spermidine; His, histamine; IAM, Institute of Applied Microbiology, The University of Tokyo, Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Osaka, Japan; JCM, Japan Collection of Microorganisms, RIKEN, Saitama, Japan; GUNMA, Gunma University School of Medicine, Maebashi, Japan; TOKAI, Tokai University School of Medicine, Kanagawa, Japan; ATCC, American Type Culture Collection, Rockville, USA; ND, not detected (< 0.01). The names of the strains which have no standing in nomenclature appear in inside quotation marks.

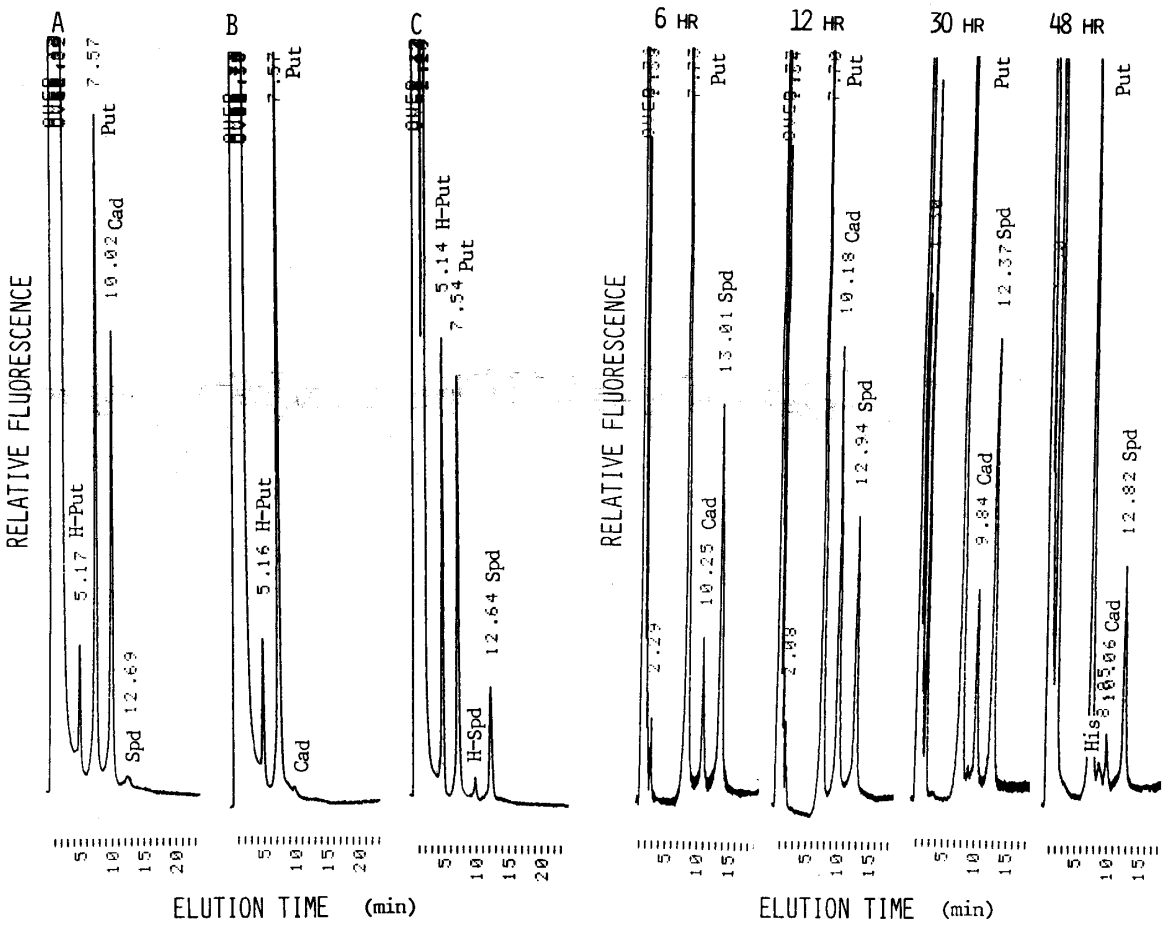

Fig. 1. HPLC separation of polyamines in *Pseudomonas cepacia* GUNMA (A), *Alcaligenes eutrophus* IAM 12627 (B) and *Comamonas acidovorans* IFO 13582 (C) cultured for 24 hr. Abbreviations for polyamines are shown in Table 1.

Fig. 2. HPLC separation of polyamine in *Pseudomonas aeruginosa* PA01 (TOKAI) cultured for 6 hr, 12 hr, 30 hr and 48 hr. Abbreviations for polyamines are shown in Table 1.

Table 2. Cellular concentrations of polyamines in *Pseudomonas aeruginosa* PA01 at different culture times.

Strain	Culture time (hr)	Polyamine (nmol/mg protein) Put	His	Cad	Spd
TOKAI	6	145	ND	7.5	3.0
	12	46	ND	9.8	8.6
	30	65	ND	3.7	21
	48	53	0.3	1.0	7.9
GUNMA (Iyobe)	30	78	ND	3.5	25
GUNMA (Inoue)	30	94	ND	3.0	29
GUNMA 13-15	30	54	ND	3.9	15
GUNMA 13-18	30	99	ND	4.7	30

Abbreviations: ND, not detected (<0.1); Others are shown in Table 1.