DISTRIBUTION OF HYDROXYPOLYAMINES, AMINO- PROPYLCADAVERINE, AND SPERMINE IN THERMOPHILIC, HYDROGEN- OXIDIZING PSEUDOMONADS

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A slightly thermophilic hydrogen bacterium, “*Pseudomonas hydrogenothermophila*,” was found to contain 2-hydroxyputrescine, 2-hydroxy-spermidine, diaminopropane, putrescine, cadaverine, spermidine, aminopropylcadaverine and spermine. A mesophilic hydrogen-oxidizing pseudomonad, “*Pseudomonas hydrogenovora*” contained putrescine, 2-hydroxyputrescine, and spermidine. The polyamine distribution pattern of the latter is rather common in the beta subclass of *Proteobacteria*. The occurrence of 2-hydroxyspermidine, aminopropylcadaverine and spermine is unique in the thermophile.

Pseudomonas species are located on three rRNA branches; rRNA superfamilies I+II, III, and IV correspond to the gamma, beta, and alpha subclass of the *Proteobacteria*, respectively (5,15,17). Nearly identical polyamine distribution patterns are found in the species belonging to the same subclasses. The alpha subclass contains putrescine, and homospermidine (the first type) and the beta subclass 2-hydroxyputrescine, putrescine, and spermidine (the second type). In fluorescent pseudomonads belonging to the gamma subclass, where genus-specificity of polyamine patterns emerged, putrescine, cadaverine, and spermidine

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were found as the main components (the third type).

We isolated two thermophilic hydrogen bacteria, strain TH-1 and TH-4, growing at 50°C, which were tentatively classified as "*Pseudomonas hydrogenothermophila*" TH-1 and TH-4 (6, 7, 18), and a mesophilic, amylolytic hydrogen bacterium, strain 9-5, as "*Pseudomonas hydrogenovora*" (9-5) (11). Polyamines of these novel members of facultative chemolithotrophic hydrogen pseudomonads were analyzed in order to determine whether they serve as a chemotaxonomic marker and to evaluate their taxonomic positions on the basis of polyamine distribution patterns.

MATERIALS AND METHODS

Cultures of the three strains were performed autotrophically in the inorganic Hirsch-basal medium under the gas phase, ($H_2-O_2-CO_2 = 7:1:1$) and heterotrophically in the medium supplemented with 0.1% sodium pyruvate (6) or in synthetic 199 Medium (for mammalian cell culture, polyamine-free, Flow Laboratory, Irvine, Scotland). Static culture was carried out at 50, 45, 40 or 30°C. Cells were harvested at the logarithmically growing phase or stationary phase. 2-Hydroxyputrescine was synthesized by ammoniolysis of 1,4-dibromo-butan-2-ol (19). 2-Hydroxyspermidine and 3-hydroxyspermidine were supplied by Dr. C. Hurwitz of the Veterans Administration Medical Center, Albany, New York. Amino-propylcadaverine was synthesized in our laboratory. After centrifugation of the cultures, pellets of organisms were homogenized in equal volumes of cold 1.0N $HClO_4$. Polyamines in perchloric acid extracts were analyzed by high-performance liquid chromatography (HPLC) on a column of cation-exchange resin (Kyowa Seimitsu 62210FK) using 0.35N potassium citrate buffer system (13). Samples were also analyzed after periodate oxidation with 0.1 M sodium metaperiodate in 0.67 M NaOH for 1 h at 56°C (16). Gas chromatography (GC) was performed on a GC-9A gas chromatograph (Shimadzu Co. Ltd.) after heptafluorobutyrylation of the polyamine samples (14). The identity of polyamines was confirmed by GC-mass spectrometry (GC-Mass) on a JMS-DX 300 (JEOL Co. Ltd.) (14). For an assay of amino acid decarboxylation, the cell lysates were added to the reaction mixture containing amino acids and then incubated as described previously (12). The amounts of polyamines produced during incubation were assayed by HPLC.

RESULTS

Eight polyamine peaks corresponding to 2-hydroxyputrescine, diaminopropane, putrescine, hydroxyspermidine, cadaverine, spermidine, aminopropylcadaverine and spermine were detected on the HPLC chromatograms of "*P. hydrogenothermophila*" TH-1 and TH-4 grown at their optimum growth temperature of 50°C (Fig. 1). Two peaks corresponding to 2-hydroxyputrescine and hydroxyspermidine disappeared upon periodate oxidation of the polyamine samples. It has been shown

Fig. 1. HPLC analysis of polyamines of "*Pseudomonas hydrogenothermophila*" TH-1 grown in 199 Medium at 30°C, harvested in logarithmically growing phase (A) at 50°C, harvested in logarithmically growing phase (B) at 50°C, harvested in stationary phase (C), and polyamines of C after the periodate oxidation (D). Abbreviations: H-Put, 2-hydroxyputrescine; H-Spd, 2-hydroxyspermidine; Dap, diaminopropane; Put, putrescine; Cad, cadaverine; Spd, spermidine; Spm, spermine; APCad, aminopropylcadaverine; H-APCad, homospermidine.

that diaminopropane is produced from 3-hydroxyspermidine by such oxidation (16). Since the diaminopropane peak did not increase in height after the treatment, the polyamine in the hydroxyspermidine fraction was shown to be 2-hydroxyspermidine. Eight polyamines were also separated by GC, as shown in Fig. 2, and identified by GC-Mass analysis. Thermospermine, an isomer of spermine, was not detected by GC analysis. When the organisms were grown at 30°C, the levels of aminopropylcadaverine and spermine were lower than at 50°C. When the hydrogen bacteria TH-1 and TH-4 were cultured autotrophically under $H_2/O_2/CO_2$ gas in the inorganic medium or heterotrophically in the medium supplemented with pyruvate or 199 Medium, then similar polyamines were distributed in the organisms (Table 1). Appreciable amounts of diaminopropane were found in the cultures harvested at stationary phase. The activity of both ornithine and lysine decarboxylases was detected in the lysates of the two organisms. Diaminobutyric acid was not decarboxylated.

A mesophilic hydrogen-oxidizing "*P. hydrogenovora*" 9-5 grown under both autotrophic and heterotrophic growth conditions contained 2-hydroxyputrescine, putrescine, and spermidine. Spermidine was the main component of the organism

Fig. 2. GC analysis of polyamines of "*Pseudomonas hydrogenothermophila*" TH-1 grown in 199 Medium at 50°C, harvested in logarithmically growing phase. Abbreviations for polyamines are shown in Fig. 1.

grown at 40°C, the upper limit of growth temperature (Table 1). When the organism was cultured in the medium supplemented with 1 mM cadaverine at 40°C, then aminopropylcadaverine was not produced.

DISCUSSION

The first polyamine type is found in the alpha subclass (superfamily IV) that includes some *Pseudomonas* species, such as *P. diminuta*, *P. aminovorans*, "*P. azotocolligans*," "*P. compransoris*," "*P. carboxydovorans*," and *P. carboxydohydrogena* (1,3,8). The latter three species are carboxydotrophic bacteria.

The *Pseudomonas* species, such as *P. solonacearum*, *P. caryophylli*, *P. cepacia*, *P. marginata*, and "*P. thermocarboxydovorans*," which have the second polyamine distribution pattern, belong to the beta subclass (group III) (3,8). Four hydrogen-oxidizing *Hydrogenophaga*, *H. flava*, *H. palleronii*, *H. pseudoflava*, and *H. taeniospiralis*, formerly classified in *Pseudomonas*, belonging to the same subclass have this polyamine type (20). Our results show that the mesophilic hydrogen eubacterium "*P. hydrogenovora*" 9-5 and the slightly-thermophilic hydrogen eubacterium "*P. hydrogenothermophila*," strain TH-1 and TH-4 (formerly "*Flavobacterium autothermophilum*" TH-4(7,18), belong to this subclass. 2-Hydroxyspermidine, aminopropylcadaverine, and spermine were preferentially

Table 1. Cellular concentration of polyamines in hydrogen pseudomonads.

	Medium	Temp.	Phase	Polyamines ($\mu\text{mol/g}$ wet weight)							
				H-Put	Dap	Put	H-Spd	Cad	Spd	APCad	Spm
“ <i>Pseudomonas hydrogenothermophila</i> ” TH-1 IFO 14978	199	30	L	1.18	0.02	0.60	0.04	0.01	0.52	ND	0.04
	199	50	L	0.55	0.02	0.14	0.16	0.02	2.74	0.09	0.60
	199	50	S	1.54	0.45	0.08	0.46	1.00	0.40	0.54	2.28
	Pyruvate	50	S	0.51	ND	2.00	0.02	0.02	0.34	ND	0.02
	H ₂ /O ₂ /CO ₂	50	S	1.32	ND	0.80	0.02	0.02	0.54	ND	0.02
“ <i>Pseudomonas hydrogenothermophila</i> ” TH-4 IFO 14593	199	30	L	0.83	ND	0.70	0.02	0.02	2.00	ND	0.08
	199	50	S	2.50	0.55	0.10	0.40	0.18	1.00	0.09	1.84
	Pyruvate	50	S	0.66	ND	2.05	0.02	0.04	0.50	ND	0.02
	H ₂ /O ₂ /CO ₂	50	S	0.94	ND	0.56	0.02	0.04	0.28	ND	0.02
“ <i>Pseudomonas hydrogenovora</i> ” 9-5 DSM 1749	199	30	S	0.90	ND	3.00	ND	ND	0.80	ND	ND
	199	40	S	0.01	ND	0.08	ND	ND	4.00	ND	ND
	H ₂ /O ₂ /CO ₂	30	S	0.40	ND	1.55	ND	ND	3.24	ND	ND

The names of the strains which have no standing in nomenclature appear inside quotation marks. Abbreviations: IFO, Institute for Fermentation, Osaka, Japan; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Federal Republic of Germany; 199, synthetic 199 Medium; Pyruvate, Hirsch-basal medium supplemented with sodium pyruvate; H₂/O₂/CO₂, Hirsch-basal medium under H₂, O₂, and CO₂ gas phase; Temp., culture temperature (°C); L, logarithmically growing phase; S, stationary phase; ND, not detected (<0.005). Abbreviations for polyamines are shown in Fig. 1.

produced in the two thermophiles grown at higher temperatures, suggesting that the occurrence of the three polyamines closely coupled with their thermophily. Mesophilic "*P. hydrogenovora*" containing 2-hydroxyputrescine did not form its aminopropyl derivative, 2-hydroxyspermidine, as do most mesophilic pseudomonads belonging to the beta subclass. Another slightly thermophilic species, "*P. thermocarboxydovorans*," a carbon monoxide-utilizing pseudomonad growing at 50°C contained 2-hydroxyspermidine as a major polyamine in addition to putrescine, 2-hydroxyputrescine, and spermidine but not spermine (8). The second polyamine type in pseudomonads is further separated on the basis of composition of triamines (2-hydroxyspermidine and aminopropylcadaverine) and tetraamine (spermine).

Pseudomonas species belonging to the gamma subclass (group I + II), such as *P. alcaligenes*, *P. aeruginosa*, *P. chlororaphis*, *P. fluorescens*, *P. mendocina*, and *P. putida*, contained diaminopropane in addition to putrescine, cadaverine, and spermidine (the third polyamine distribution type) (2-4).

Taken all together, their polyamine synthetic activity would not be correlated with their hydrogen- or carbon monoxide-oxidizing activity and rather would be coupled with their thermophily in same taxonomic group (subclass) of pseudomonads. The thermophilic pseudomonad tested in this report was the third eubacterium containing the novel triamine aminopropylcadaverine, which was already found in "*Halococcus acetoinfaciens*" (10) and *Paracoccus denitrificans* (9).

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