Short Communication

ABSENCE OF CELLULAR POLYAMINES IN GRAM-POSITIVE ANAEROBIC COCCI AND LACTIC ACID BACTERIA

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Polyamine distribution profiles serve as a phenotypic chemotaxonomic marker of Gram-negative eubacteria within the class *Proteobacteria*. Distribution patterns of diaminopropane, putrescine, hydroxyputrescine, cadaverine, spermidine, nor-spermidine and homospermidine are closely associated with phylogenetic relationships among the genera belonging to the *Proteobacteria* (1,8,9). Polyamine analysis of 14 genera of aerobic Gram-positive cocci indicated also chemotaxonomic significance of cellular polyamine composition: presence of putrescine, cadaverine, spermidine and/or agmatine and absence of polyamines were species or genus specific in the aerobes (5). Gram-positive aerobic bacilli belonging to the genus *Bacillus* ubiquitously contained putrescine and spermidine (6,7). Spermine in addition to putrescine and spermidine restrictedly distributed in thermophilic species of the genus *Bacillus* (6,7). In the present study, polyamines of anaerobic Gram-positive cocci belonging to the genera *Peptococcus*, *Peptostreptococcus*, *Streptococcus*, *Lactococcus* and *Enterococcus* and Gram-positive lactic acid bacteria belonging to the genera *Atopobium*, *Carnobacterium*, *Lactobacillus*, *Leuconostoc* and *Weissella* were analyzed.

Several culture media were used to compare polyamine profiles under different growth conditions. The anaerobic cocci were anaerobically cultivated at 35°C in polyamine-free synthetic 199 medium (Nissui Pharmaceutical Co., Tokyo, Japan),

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Abbreviations: IAM, IAM Culture Collection, Center for Cellular and Molecular Research, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Osaka, Japan; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research (RIKEN), Wako, Japan; GIFU, Department of Microbiology, Gifu University School of Medicine, Gifu, Japan; ATCC, American Type Culture Collection, Rockville, MD, U.S.A.

pH 7.0, using for mammalian cell culture, the 199 medium supplemented with 0.03% L-cysteine and 0.1% Tween 80 (199-CysTW) and/or GAM broth (Nissui Pharmaceutical Co.) prepared for the culture of anaerobes, using Anaerobic Jar KJ-1 (Tomy Seiko, Tokyo, Japan). Lactic acid bacteria were microaerobically, statistically grown at 30 or 35°C in the 199 medium supplemented with 0.01% L-cysteine, 0.1% lactose and 0.1% Tween 80 (199-CysLacTW), the MRS broth (Difco Lab., Detroit, MI, U.S.A.) supplemented with 0.01% L-cysteine, 1% lactose and 0.5% liver powder (Kyokuto Pharmaceutical Co., Tokyo, Japan) (MRS-CysLacLp) and/or GAM broth. Concentrations of polyamines detected in GAM and MRS media containing polyamines are shown in Table 1. The organisms at the stationary phase were harvested by centrifugation. The packed cells were homogenized in equal volumes of cold 1 M HClO₄ to extract polyamines. For complete recovery of cellular polyamines, packed cells were hydrolyzed directly with 6 M HCl at 110°C for 24 h. The polyamines in the HClO₄-extracts and HCl-hydrolyzates were analyzed by high-performance liquid chromatography (HPLC) on an L600 High-Speed Liquid Chromatograph (Hitachi Co., Tokyo, Japan) using a column (4 mm I.D. × 50 mm) of cation-exchange resin (Hitachi #2619F) kept at 70°C. The elution system consisted of a linear gradient of two buffers, 0.045 M sodium citrate/0.06 M citric acid/0.064 M NaCl and 0.20 M sodium citrate/2.0 M NaCl. Polyamines eluted by HPLC were detected by fluorometry on a F1050 Fluorescence Spectrometer (Hitachi Co.) after heating with *o*-phthalaldehyde reagent, as shown in the previous report (5).

When the anaerobic cocci were grown in the polyamine-free synthetic 199

Table 1. Cellular concentration of polyamines in *Peptococcus*, *Peptostreptococcus*, *Streptococcus*, *Lactococcus*, *Enterococcus*, *Atopobium*, *Carnobacterium*, *Lactobacillus*, *Leuconostoc* and *Weissella* grown in polyamine-containing media.

	Polyamine (μmol/g wet cell)				
	Medium	Put	Cad	Spd	Spm
<i>Peptococcus niger</i> ATCC 27731 ^T	GAM	ND	ND	0.01	0.01
<i>Peptostreptococcus productus</i> JCM 1471 ^T	GAM	ND	0.02	0.09	0.15
<i>Streptococcus pyogenes</i> JCM 5674 ^T	GAM	0.05	0.18	1.50	0.03
<i>Lactococcus lactis</i> subsp. <i>cremoris</i> IFO 3427	GAM	ND	0.07	2.50	0.25
<i>Enterococcus faecalis</i> JCM 5803 ^T	GAM	0.01	0.02	0.95	0.82
<i>Atopobium rimae</i> IFO 15546 ^T	GAM	ND	ND	0.01	0.01
<i>Carnobacterium divergens</i> JCM 5816 ^T	GAM	0.01	0.03	0.44	0.09
<i>Lactobacillus acidophilus</i> IAM 10074	MRS-CysLacLp	ND	0.02	0.45	0.04
<i>Leuconostoc lactis</i> JCM 6123 ^T	GAM	ND	0.01	0.75	0.57
<i>Weissella confusus</i> JCM 1093 ^T	GAM	ND	0.01	0.92	0.04
199 medium (μmol/l)		ND	ND	ND	ND
GAM broth (μmol/l)		0.15	0.10	0.06	0.01
MRS broth (μmol/l)		0.01	0.03	0.05	0.01

Put, putrescine; Cad, cadaverine; Spd, spermidine; Spm, spermine; ^T, type strain; ND, not detectable (<0.005 μmol/g wet cell).

medium supplemented with cysteine, lactose and/or Tween 80, the genus *Peptococcus*, containing the single species *P. niger* ATCC 27731^T and all species of *Peptostreptococcus* (14 species) (*P. tetradius* JCM 1964^T, *P. prevotii* JCM 6490^T, *P. hydrogenalis* JCM 7635^T, *P. productus* JCM 1471^T, *P. anaerobius* GIFU 7882^T, *P. asaccharolyticus* GIFU 7656^T, *P. indolicus* GIFU 7848^T, *P. magnus* GIFU 7881^T, *P. micros* GIFU 7824^T, *P. lacrimalis* GIFU 7667^T, *P. lactolyticus* GIFU 8586^T, *P. parvulus* GIFU 7762^T, *P. vaginalis* GIFU 7732^T, *P. barnesae* GIFU 12145^T), *Streptococcus* (8 species) (*S. pyogenes* JCM 5674^T, *S. mutans* JCM 5705^T, *S. salivarius* subsp. *salivarius* JCM 5707^T, *S. salivarius* subsp. *thermophilus* IFO 13957, *S. sanguis* JCM 5708^T, *S. uberis* JCM 5709^T, *S. bovis* JCM 5802^T, *S. equinus* JCM 7879^T), *Lactococcus* (4 species) (*L. lactis* subsp. *cremoris* IFO 3427, *L. lactis* subsp. *hordniae* JCM 1180^T, *L. lactis* subsp. *lactis* JCM 5805^T, *L. raffinolactis* JCM 5706^T), and *Enterococcus* (5 species) (*E. faecalis* JCM 5803^T, IFO 3971, IFO 12965, *E. faecium* JCM 5804^T, *E. casseliflavus* JCM 5675, *E. durans* IFO 13131, *E. hirae* IFO 3181^T) tested in this study were devoid of polyamines.

Lactic acid bacteria belonging to *Atopobium* (2 species) (*A. rimae* IFO 15546^T, *A. minutum* JCM 1118^T), *Carnobacterium* (2 species) (*C. divergens* JCM 5816^T, *C. piscicola* JCM 5848^T), *Lactobacillus* (14 species) (*L. acidophilus* IAM 10074, *L. animalis* IAM 13547^T, *L. batatas* IAM 10063, *L. brevis* IAM 1082, *L. casei* subsp. *casei* IAM 12473^T, *L. casei* subsp. *rhamnosus* IAM 1118^T, *L. delbrueckii* subsp. *bulgaricus* IAM 12472^T, *L. delbrueckii* subsp. *delbrueckii* IAM 12474^T, *L. delbrueckii* subsp. *lactis* IAM 12476^T, *L. fermentum* IAM 1148, *L. helveticus* IAM 12475, *L. japonicus* IAM 10068, *L. plantarum* IAM 12477^T, *L. sake* IAM 10077), *Leuconostoc* (5 species) (*L. lactis* JCM 6123^T, *L. oneos* JCM 6125^T, *L. mesenteroides* subsp. *cremoris* IAM 1087, *L. mesenteroides* subsp. *dextranicum* IAM 1122, *L. mesenteroides* subsp. *mesenteroides* IAM 13004^T) and *Weissella* (6 species) (*W. confusus* JCM 1093^T, *W. halotorelans* JCM 1114^T, *W. hellenica* IFO 15553^T, *W. kandleri* JCM 5817^T, *W. minor* JCM 1168^T, *W. viridescens* IAM 13546^T) grown in the 199 medium supplemented with cysteine, lactose and Tween 80 also lacked polyamines.

The absence of cellular polyamines in the organisms in which polyamines were not detected in the HClO₄-extracts was confirmed by polyamine analysis of the HCl-hydrolyzates of the packed cells. Spermidine and spermine were not produced by the organisms grown in the polyamine-free synthetic media supplemented with putrescine. The all organisms grown either in GAM medium or MRS-CysLacLp medium contained putrescine, cadaverine, spermidine and spermine, suggesting uptake of these polyamines from the media. Typical data are shown in Table 1. A higher polyamine level was found in the cultures with GAM medium rather than with MRS medium. *P. niger* and *A. rimae* cultivated in polyamine-containing GAM medium scarcely contained polyamines. In some strains of *Lactobacillus*, polyamines were almost undetectable in the culture with MRS-CysLacLp medium. The uptake levels of polyamines from the same polyamine-containing medium showed species-specific variation. Tentative polyamine analysis of some *Streptococ-*

cus and *Lactobacillus* grown in polyamine-containing media have also shown the occurrence of some cellular polyamines at low levels (6).

The new genus *Atopobium* was located intermediate between cocci and bacilli of lactic acid bacteria, however, phylogenetically divergent from streptococci, lactococci and lactobacilli (4). Atypical lactobacilli and some *Leuconostoc*-like organisms were reclassified in a new genus *Weissella* (3). Some catalase-negative, asporogenous, rod-shaped lactic acid bacteria were classified in a new genus *Carnobacterium* (2). These results indicate the ubiquitous absence of ornithine-, lysine- and arginine-decarboxylase activities to produce putrescine, cadaverine and agmatine in various Gram-positive anaerobic cocci and lactic acid bacteria.

Cellular polyamines were shown to be absent in some Gram-positive aerobic cocci including the genera *Aerococcus*, *Alloiococcus*, *Helicococcus*, *Kineococcus*, *Pediococcus* and *Tetragenococcus* grown in polyamine-free culture media (5). Other Gram-positive aerobic cocci belonging to the genera *Planococcus*, *Marinococcus*, *Salinicoccus*, *Stomatococcus* and *Deinococcus* synthesized putrescine, spermidine and/or agmatine (5). Gram-positive *Bacillus* species ubiquitously contained putrescine and spermidine (6,7). The absence of polyamine synthetic ability serves as a phenotypic property of some Gram-positive aerobic cocci, major Gram-positive anaerobic cocci and Gram-positive lactic acid bacteria. The phylogenetic relation between the three Gram-positive eubacterial groups is suggested by the ubiquitous absence of polyamines, however, polyamine analysis is less usefulness as a chemotaxonomic marker within Gram-positive anaerobic cocci and lactic acid bacteria.

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