

THE POLYAMINE CELL COMPOSITION AS A CHEMOTAXONOMIC MARKER IN LACTIC ACID BACTERIA IDENTIFICATION

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Summary

Analysis of the cell polyamines - cadaverine, putrescine, spermine and spermidine of 11 newly isolated strains of lactic acid bacteria from different regions of West Bulgaria was performed. The obtained data were compared with the physiological and biochemical characteristics of these strains, so that the polyamine analysis could be used as a taxonomic criterion. The results showed that spermidine was the main polyamine in all tested cultures. Spermine and putrescine were found in small quantities in two strains, whereas cadaverine - in none of the studied strains. Spermidine values were high and varied in the different strains and showed good correlation with their physiological and biochemical characteristics. On the basis of the obtained results a conclusion was made that the polyamine analysis can serve as a chemotaxonomic criterion only on the strictly standardized cultivation conditions and in the presence of data-base for qualitative and quantitative composition of the corresponding type cultures.

Introduction

Investigations on microbial chemistry has shown that cell component analysis can be used in bacterial classification and identification [2]. Recently, the polyamine cell composition is used as a chemotaxonomic marker in addition to determination of cell wall composition, protein patterns, fatty acids and enzyme characterisation [6, 7, 8, 9, 15, 16, 11, 10].

Polyamines as putrescine, spermidine, spermine and cadaverine are polycationic compounds which are widely distributed in nature. Several lines of evidence show that these polyamines are essential for growth and the regulation of macromolecular synthesis in almost all organisms [1]. Putrescine and spermidine are the main polyamines present in most prokaryotic organisms: eubacteria, archaebacteria and cyanobacteria [5]. Considerable amounts of spermidine have been found in thermophilic bacilli [11], thermophilic actinomycetes [6], acetobacteria [10] and agrobacteria [16]. Not considerable quantities of it were found in lactic acid bacteria [7]. Cadaverine is also synthesized but not by all mi-

croorganisms. Even if spermine is detected in certain eubacteria, it does not necessarily mean that they synthesize spermine, because polyamines can be taken up from culture media by growing bacteria. Trace amounts of spermine can be detected even in some eubacteria that lack spermine synthetic activity such as *Escherichia coli* [9] and *Staphylococci* [13].

Recently the so called "unusual" polyamines have been found in various microorganisms. Norspermidine is synthesized by halophilic strains of *Vibrionaceae* family [18] while symhomospermidine - by photosynthetic bacteria [4, 5], nitrogen-fixing bacteria [5, 12] and sulphur-oxidising bacteria [5]. Strains from *Thermus* [8] and *Clostridium* [11] genera possess norspermidine and thermospermidine.

The present work focuses on the application of polyamine analysis for a more complete characterization of newly isolated strains of lactic acid bacteria.

Materials and Methods

Microorganisms and media. Eleven newly isolated strains of lactic acid bacteria from different starters named at the present stage of investigation as N-9-11, R-26-3, R-11-5, P-1, R-11, F-29-4, R-29, R-9-1, R-14-10, F-35-3 and F-1-9 had been used for analysis of the cell polyamines.

The strains were cultivated on MRS medium containing (g/l): bactopectone - 10, meat extract - 10, yeast extract - 5, glucose - 20, diamonium citrate - 2g, $MgSO_4 \cdot 7H_2O$ - 0.2, K_2HPO_4 - 2, CH_3COONa - 5, $MnSO_4 \cdot 7H_2O$ - 0.05, Tween-80 - 2.1ml (pH of the media was adjusted to 5.2). Three-stage cultivation was performed: 1) The freeze-dried strains were suspended in 3 ml skim milk and cultivated for 24 h; 2) 0.1 ml of this first culture was inoculated in 3 ml MRS liquid medium for 24 h; 3) 6 ml of the second culture were inoculated in 250 ml MRS medium for 48 h. The cultivation temperatures of each strain were shown in Table 1. The biomass was separated by centrifugation at 8 000 rpm, 4°C on Beckman JA-21 centrifuge and triply washed with physiological solution under the same conditions.

Physiological and biochemical tests. Colonial morphology was examined on isolated colonies grown on MRS agar for 2 - 4d. Cellular morphology was examined in Gram-stained smears. The fermentation of different sugars was studied in liquid MRS medium without meat extract and glucose and with 0.05% (w/v) chlorphenol red. The

sterilized by filtration sugar and alcoholsolutions were added in the medium to a final concentration of 1%. The test cultures cultivation was performed at optimal for the growth of the different strains temperatures. The presence of metachromatic grains in the cells was detected microscopically after staining with methylene blue, and milk coagulation - after inoculation of the strains in sterile skim milk.

The growth of the cultures in the presence of 2% NaCl was established at temperatures 15 and 45°C.

The tests for NH_3 production from arginine, gelatinase and catalase activities had been performed according to the methods described in [3].

Bergey's manual [2] was used for identification of the strains and *Lactobacillus bulgaricus* NBIMCC 1382 was used as a referent strain.

Polyamine analysis. 1 g fresh biomass was washed with distilled water and homogenized with equal volume 10% 3-chloroacetic acid. The polyamine extraction had been performed for 2 h at 4°C, followed by centrifugation at 15 000 rpm for 20 min at 4°C. The supernatant was lyophilized and after solubilization of the dry matter in 0.6 ml 0.1N HCl was used for analysis. Polyamines were determined quantitatively by use of Amino acid analyzer type AAA-Prague and ninhydrin reaction.

Results and Discussion

The investigation of the polyamine pattern cannot be applied in isolation without classical taxonomic information. All of the isolated strains are Gram positive rods with rounded ends, 0.5-0.8 by about 2-9µm, occurring singly and in short chains. Growth on solid medium showed that they form R and S colonies. No catalase and gelatinase activity was observed. According to Bergey's manual some physiological and biochemical characteristics were determined. The results are presented in Table 1. Nine of the studied strains showed phenotypic characteristics close to that of *L. delbrueckii* subsp. *bulgaricus*- they ferment

only some sugars, possess metachromatic grains, do not form NH_3 from arginine, grow at temperature 45°C in medium with 2% NaCl, but growth at 15°C was not observed. Strain F-1-9 showed high phenotypic similarities with *L. delbrueckii* subsp. *bulgaricus*, with exception of its ability to metabolize maltose. This places the strain close to *L. delbrueckii* subsp. *lactis* and *L. casei* subsp. *casei*. The obtained results for strain P-14-10 (positive characteristics for esculin, maltose, sucrose and NH_3 from arginine) distinguish it from the other strains and include it in the group of *L.*

Table 1. Some physiological and biochemical characteristics of newly isolated lactic acid bacteria strains

Strains	Characteristics ^a																							
	ara	cel	esc	fru	gal	glu	lac	mal	mnt	man	mel	raf	rbs	sac	xyl	rha	mcg	15°	45°	mca	gat	gel	NaCl	NH ₃
N-19-11	-	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	+	-	-	+	-
F-26-3	-	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	+	-	-	+	-
R-11-5	-	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	+	-	-	+	-
P-1	-	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	+	-	-	+	-
R-11	-	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	+	-	-	+	-
F-29-4	-	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	+	-	-	+	-
R-29	-	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	+	-	-	+	-
R-9-1	-	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	+	-	-	+	-
R-14-10	-	-	+	+	+	+	+	+	-	+	-	-	-	+	-	-	-	-	+	+	-	-	+	+
F-35-3	-	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	+	-	-	+	-
F-1-9	-	-	-	+	+	+	+	+	-	+	-	-	-	-	-	-	+	-	+	+	-	-	+	-
Ref.St. ^b	-	-	-	+	-	+	+	-	-	+	-	-	-	-	-	-	+	-	+	+	-	-	+	-

^a - ara - arabinose, cel - cellobiose, esc - esculine, fru - fructose, gal - galactose, glu - glucose, lac - lactose, mal - maltose, mnt - mannitol, man - mannose, mel - melibiose, raf - raffinose, rbs - ribose, sac - saccharose, xyl - xylose, rha - rhamnose, mcg - meta chromatic grains, 15° - growth at 15°C, 45° - growth at 45°C, mca - milk coagulating activity, gat - catalase, gel - gelatine, NaCl - growth on 2% NaCl, NH₃ - ammonia; ^b - Ref.St. - referent strain *Lactobacillus bulgaricus* (NBIMCC 1132).

Table 2. Polyamine cell composition of newly isolated lactic acid bacteria strains

Strains	Cultivation temperature	Polyamines ^a (μmol/g fresh biomass)			
		Pn	Cd	Sd	Sn
N-19-11	37°C	-	-	0.021	-
F-26-3	37°C	-	-	0.023	-
R-11-5	37°C	-	-	0.021	-
P-1	37°C	-	-	0.022	-
R-11	28°C	-	-	0.013	-
F-29-4	45°C	-	-	0.021	-
R-29	28°C	-	-	0.029	-
R-9-1	37°C	-	-	0.055	-
R-14-10	37°C	0.003	-	0.086	0.002
F-35-3	37°C	-	-	0.027	0.006
F-1-9	37°C	-	-	0.216	-
Ref.St ^b	45°C	-	-	0.012	0.002

^a - Pn - putrescine, Cd - cadaverine, Sd - spermidine, Sn - spermine;
^b - Ref.St. - *Lactobacillus bulgaricus* (NBIMCC 1132)

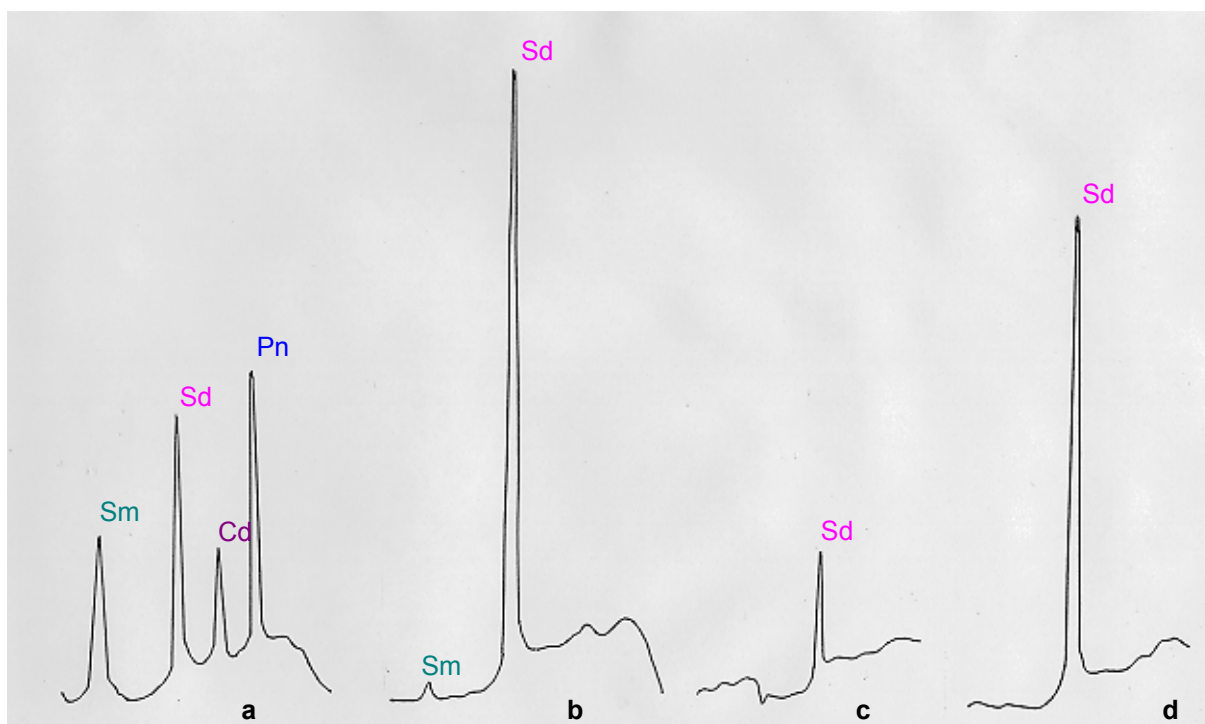


Fig. 1. Chromatograms off polyamines of some lactic acid bacteria. Standard (a) and strains R-14-10 (b), F-26-3 (c) and F-1-9 (d): spermine (Sm), spermidine (Sd), cadaverine (Cd) and putrescine (Pn).

delbrueckii subsp. *lactis*. Further investigations are necessary for correct classification of these strains into corresponding species.

For a more complete characterization of newly isolated strains of lactic acid bacteria and to evaluate the possibility to use as taxonomic criterion, polyamine analysis was carried out. The obtained results showed that spermidine is the main polyamine in all tested cultures (Table 2). Spermine appeared only in R-14-10 and F-35-3, while putrescine - in R-14-10. Spermidine values in N-19-11, F-26-3, R-11-5, P-1, F-29-4, R-29, F-35-3 and R-11 did not vary significantly: 0.013 - 0.027 mol/g fresh biomass. Chromatograms of some of the strains are presented on Fig. 1a, b and c.

Guirard and Snell [4] showed that spermine was present in various lactic acid bacteria after analyzing polyamines by a bioassay procedure. The trace amount of spermine has also been detected in these organisms in the study of Hamana et al. [7]. These authors suggested that this seems to be due to the uptake or contamination from the medium containing peptone, yeast extract or tryptose. Our results did not confirm also the spermine biosynthesis by lactic acid bacteria. Small quantities were found only in R-14-10 and F-35-3.

Putrescine was not present in the cells of the studied strains with the exception of R-14-10. Cadaverine was not found in anyone of them (Table 2). Hamana et al. [7] had found considerable amount of this polyamine only in

L. acidophilus which probably was due to induction of the cadaverine biosynthesis by the specific growth conditions.

The results of the polyamine analysis of 11 lactic acid bacteria strains showed that species differentiation could be done using quantitative determination of spermidine. Its values were high and variable for the strains belonging to the different species. Comparing our results for the spermidine with those of Hamana et al. [7], we found out that N-19-11, F-26-3, R-11-5, P-11, F-29-4, R-29, P-9-1, F-35-3 belonged to the group of *L. delbrueckii* subsp. *bulgaricus*; F-1-9. Strains F-1-9 and R-14-10 showed differences not only in their physiological and biochemical characteristics but also in their polyamine content (0.086 mol/g fresh biomass for R-14-10 and 0.216 mol/g for F-1-9). The insufficient information in literature about the polyamine composition of the corresponding type strains did not allow us to classify R-14-10 and F-1-9 into a certain group.

Summarizing the data of the present investigation and those in the literature it can be concluded that the polyamine analysis gives reliable proofs for the physiological status of certain species of the *Lactobacillus* genera. Strict standardization of the cultivation condition and presence of data-base for the quantitative and qualitative polyamine composition of the corresponding type cultures should be done in order to use it as a chemotaxonomic marker.

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