PE4.111 Phenotypisation of LAB Strains Isolated from 'Uzicka' Sausage 404.00

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Phenotypisation of LAB strains Isolated from „Uzicka" Sausage Slavica Veskovic, Branka Borovic, Branko Velebit, Dragica Karan, Lazar Turubatovic, Dragojlo Obradovic and Djordje Okanovic Abstract— Paper describes basic phenotypic characteristics of indigenous strains of lactic acid bacteria (LAB) which have been isolated at different stages of ripening of fermented Serbian sausage "Uzicka" sausage. A total of 150 strains of LAB have been isolated and characterized using standard microbiological methods. An experiment was repeated three times. Final confirmation of LAB strains was performed using API 50 CHL test. Out of 11 isolated LAB strains the most dominant were Lb. delbrueckii ssp. delbrueckii, Lb. plantarum, Lb. delbrueckii ssp. bulgaricus, Lb fermentum and Lb. curvatus. Following metabolic characteristics of isolated LAB strains were also investigated: gas production from glucose, slime formation, growth at different temperatures (4, 10, 15, 37 and 45°C), cell morphology, catalase reaction. At the end of 21-day ripening of "Uzicka" sausage sensory properties were also evaluated.

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Index Terms—fermented sausages, uzicka sausage, LAB, phenotypic characterisics.

I. INTRODUCTION

Lactic acid bacteria (LAB) as well as Micrococcaceae strains are important microorganisms used as starter cultures in meat fermentations. Their addition to meats may improve safety and stability of the product extending the shelf life and provides diversity resulting in new sensory properties as well as health benefits by probiotic characteristics as reported in a recent review [8].The most frequently isolated lactic acid bacteria from dry sausages processed with different technologies are Lactobacillus sakei, L. curvatus and L. plantarum [1, 2, 3, 4]. The isolation and selection of lactic acid bacteria which can be used as starter cultures in meat fermentation present a considerable challenge to standardization and management of quality of dry fermented sausage. Lactic acid bacteria originating from fermented meats are specially adapted to the ecology of meat fermentation [3]. In addition, they control the entire fermentation and ripening processes, inhibiting the growth of spontaneous lactic acid bacteria [3]. Production of fermented meat products is result of metabolic activity of inherent and within meat-adapted LAB. Quality of traditionally fermented sausage is influenced by many factors such as selection of raw material, metabolic activity of epiphytic flora and physic-chemical properties developed during ripening, smoking and drying of meat [5, 6]. Until now there were no comprehensive investigations in Serbia regarding diversity and characteristics of indigenous microflora in traditionally fermented sausages which resulted in poor data about possible appliance in food industry. In order to protect the traditional aspect of these products, it is essential to understand the microbial ecology during fermentation by studying the dynamic changes that occur and to select autochthonous starter cultures that can be used in the production [7]. Fermentation with autochthonous starter cultures allows the production of sausages with lower safety risks than the ones naturally fermented [8]. Aim of this paper was to develop national collection ("bank") of autochthonous LAB strains which could present future basis for production of domestic starter cultures.

II. MATERIALS AND METHODS

A. Uzicka sausage

"Uzicka" sausage was produced according to traditional household technology on Zlatibor Mountain. It was composed of beef, pork, minced beef and solid

fat at the percentage ratio 50:20:20:10. Prepared raw material was minced down to granulation size of 2-3 mm. Following additives were added: nitrite salt (2.5%), common salt (0.30%) and S-Alimenta (0.87%). Filling was stuffed into bovine small intestines. Smoking, fermentation, ripening and drying lasted 21 day.

B. Microbiological investigation

Three samples were taken at each stage of production of fermented sausage (there were three individual fermentations) and were microbiologically investigated (Day 0, 2, 4, 7, 14 and 21). A 25 g of each sample was added to 225 mL of MRD (Oxoid, United Kingdom). Samples were homogenized in stomacher (AES, France) for 90 s. A series of decimal dilutions was made and 1 mL of each dilution was pippeted in two Petri dishes and following media were poured: a) TVC-PCA (Merck, Germany) and incubated during 48-72 h at the temperature of 30°C; b) LAB-MRS agar (Oxoid, United Kingdom), double layered and incubated during 48 h at the temperature of 30°C; c) Micrococcaceae-MSA agar (Oxoid, United Kingdom), incubated during 48 h at the temperature of 30°C; d) Enterobacteriaceae-VRBD agar (Merck, Germany), incubated during 24 h at the temperature of 37°C; e) Enterococcaceae-Bile-aesculin-azide agar (Merck, Germany), incubated during 24 h at the temperature of 37°C; f) Yeasts and moulds-DG18 agar (Merck, Germany), incubated during 48-72 h at the temperature of 25°C. Standard microbiological methods were used for isolation of LAB and MIC while the final confirmation was performed using API 50 CHL and API 32 ID Staph tests.

C. Sensory analysis

A panel of 10 evaluators evaluated product after 21 day of fermentation. A quantitative-descriptive test specially adapted to this type of product was used. Following properties were evaluated: color, surface of cut, coherence, smell, fat quality, acidity, juicy, tenderness and overall impression.

III. RESULTS AND DISCUSSION

A. Diversity of technological microbiota

Lactic acid bacteria and Staphylococcus or Micrococcaceae belonging to the CNC group are considered as technological microbiota because they are involved in the development of hygienic and sensory qualities of the final product. Lactic acid bacteria are involved mainly through their acidification. Staphylococcus and Micrococcaceae contribute to the development of color and flavor in fermented meat products mainly by degrading free amino acids and inhibiting the oxidation of unsaturated free fatty acids [9, 10]. Results of investigation of variation of epiphytic flora of "Uzicka" sausage during fermentation are shown in Table 1. TVC increased until day 7 (log 6.1 ± 0.85 cfu/g) and decreased until the end of fermentation (log 5.1 ± 0.46 cfu/g). Starting LAB count (log 3.23 ± 0.13 cfu/g) increased until day 14 (log 5.68 ± 0.75 cfu/g) and mild decrease occurred. At the end of fermentation and ripening LAB count was log 5,30 ± 0.55 cfu/g. Micrococcaceae count remained unchanged until day 4 (log 3,35 cfu/g) when gradual decrease occurred (log 2,35 cfu/g). Enterococcaceae count had tendency of decrease. Initial established values were log 3.54 ± 0.47 cfu/g while after day 14 counts was less than log 1. No Enterobacteriaceae yeasts and moulds were detected after day 4 and 14, respectively. Pathogenic bacteria were not detected during production of "Uzicka" sausage.

B. LAB-carriers of lactic fermentation

LAB isolated from "Uzicka" sausage at different stages of fermentation are shown in Table 2. A total of 11 LAB strains have been isolated and characterized using standard microbiological methods. Final confirmation of LAB strains was performed using API 50 CHL test. The most dominant were Lb. delbrueckii ssp. delbrueckii, Lb. plantarum, Lb. delbrueckii ssp. bulgaricus, Lb fermentum, Lb. brevis and Lb. curvatus. These isolates make 79.9% of all the isolated strains and they are carriers of lactic fermentation and ripening of investigated sausage. Property of LAB to ferment carbohydrates to lactic acid is basic principle that determines their appliance in production of fermented meat products. Production of lactic acid is a result of glucose cleavage during glycolysis or 6-phosphogluconate/phosphoketolase reaction depending whether they are homo- or heteroferementative species [11, 12]. Homofermentative group of LAB during EMP pathway converts 1 mol of glucose into 2 mol's of lactate whereas heteroferementative group of LAB during anaerobic cleavage of glucose form lactic acid, carbon-dioxide, ethanol and half the energy than previous group [13, 14].

C. *Morphological and presumable technological properties of isolated LAB*

Morphological properties and biochemical tests performed in order to identify the isolated lactic acid bacteria are shown in Table 3. Results indicated that none of the isolates produce carbon-dioxide from glucose so it is important technological property. Furthermore all the 150 isolates acquired during three independent fermentations will be genotypically identified by partial 16S sequencing

D. *Sensory evaluation of "Uzicka" sausage*

Results of sensory evaluation showed that "Uzicka" sausage are presented on Figure 1. "Uzicka" sausage is highly valuable traditional meat product which marked off during the project. During the all three fermentations sensory properties gained high marks (above 8.5 at the scale from 1 to 10).

IV. CONCLUSION

By following trends in production of fermented meat products more and more meat processors in Serbia are using starter cultures. Since there is no commercial production of starter cultures in our country, meat industry is forced to buy them from abroad. These starter cultures are often adapted to needs of foreign markets. Therefore their appliance in domestic production doesn't result in products which are by theirs quality characteristics acceptable for domestic consumers. Problem could be solved by using LAB strains isolated from autochthonous fermented sausages as a starter cultures. These could be used for future production of domestic starter cultures which could be done independently or in cooperation with some of the recognized world companies.

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REFERENCES

[1] Hammes, W.P., Bantleon, A., Min, S., (1990). Lactic acid bacteria in meat fermentation. FEMS Microbiol. Rev., 87: 165-174.

[2] Ammor, S., Dufour, E., Zagorec, M., Chaillou, S., Chevallier, I., (2005). Characterization and selection of Lactobacillus sakei strains isolated from traditional dry sausage for their potential use as starter cultures. Food Microbiol., 22: 529-538.

[3] Hugas, M., Monfort, J.M., (1997). Bacterial starter cultures for meat fermentation. Food Chem., 59: 547-554.

[4] Schillinger, U., Lücke, F.K., (1989). Antimicrobial activity of Lactobacillus sake isolated from meat. Appl. Environ. Microbiol., 55: 1901-1906.

[5] Vesković, S., (2005). Influence of bacteriocin Leuconostoc mesenteroides E 131 and Lactobacillus sakei I 154 on Listeria monocytogenes during production of Sremska sausage, M.Sc thesis, Faculty of Agriculture, Zemun – Belgrade.

[6] Vesković, S., (2007). bacteriocin Leuconostoc mesenteroides E 131, Lactobacillus sakei I 154 and MAP on shelflife of Sremska sausage. Ph.D. dissertation, Faculty of Agriculture, Zemun – Belgrade..

[7] Rantsiou, K., & Cocolin, L. (2006). New developments in the study of the microbiota of naturally fermented sausages as determined by molecular methods: a review. International Journal of Food Microbiology, 108, 255–267.

[8] Lücke, F. K. (2000). Utilization of microbes to process and preserve meat. Meat Sci., 56, 105–115.

[9] Talon, R., & Leroy, S. (2006). Latest developments in meat bacterial starters – Chapter 16. In L. M. L. Nollet & F. Toldra (Eds.), Advanced technologies for meat processing (pp. 401–418). New York: CRC Press/Taylor and Francis Group.

[10] Talon, R., Leroy-Se´trin, S., & Fadda, S. (2004). Dry fermented sausages – Chapter 23. In Y. H. Hui, L. Meunier-Goddik, A. S. Hansen, J.Josephsen, W.-K. Nip, & P. S. Stanfield, et al. (Eds.), Handbook of food and beverage fermentation technology (pp. 397–416). New York:Marcel Dekker Inc.

[11] Kandler, O., (1983). Carbohydrate metabolism in lactic acid bacteria. Ant. van Leeuwenhoek, 49: 209-224.

[12] Axelsson, L., (1998). Lactic Acid Bacteria: Classification and Physiology. In. Salminen, S., von Wright, A. (Eds.), Lactic acid bacteria: Microbiology and Functional Aspects, 2nd Edition, Marcel Dekker Inc., New York, pp. 1-72.

[13] Ross, R.P., Morgan, S., Hill, C., (2002). Preservation and fermentation: past, present and future. Int. J. Food Microbiol., 79: 3-16.

[14] Caplice, E., Fitzgerald, G.F., (1999). Food fermentations: role of microorganisms in food production and preservation. Int. J. Food Microbiol., 50: 131-149.

Table 1. Dynamics of variation of epiphytic flora isolated from "Uzicka" sausage

Type of investigation	Day of fermentation											
	0		2		4		7		14		21	
	Ā	SD	Ā	SD	Ā	SD	Ā	SD	Ā	SD	Ā	SD
TVC	5.83	1.16	5.52	1.24	5.13	1.40	6.10	0.85	5.59	0.52	5.21	0.46
LAB	3.23	0.13	2.93	0.41	3.16	0.28	3.73	0.67	5.68	0.75	5.30	0.55
Micrococcaceae	3.15	0.30	3.40	0.35	3.09	0.36	2.65	0.16	2.63	0.55	2.35	1.29
Enterococcaceae	3.54	0.47	3.33	0.55	<1.72		<1.65		<1.0			
Enterobacteriaceae	<2.0		<2.0		<2.0							
Yeasts and moulds	3.49	0.20	2.61	0.54	2.53	0.36	1.73	0.68				

Table 2. API identification of LAB isolated from "Uzicka" sausage

Count of isolates (days)						Count of isolates (%)	API identification
0	**2**	**4**	**7**	**14**	**21**		
0	1	3	1	5	5	15 (10.0)	*Lb.fermentum*
3	0	1	1	0	1	6 (4.0)	*Ln. mesenteroides ssp. mesenteroides*
4	5	5	8	10	10	42 (28.0)	*Lb. delbrueckii ssp. delbrueckii*
2	2	2	4	2	1	13 (8.6)	*Lb.brevis*
1	3	1	2	5	2	14 (9.3)	*Lb. curvatus*
3	0	2	4	2	0	11 (7.3)	*Lc. lactis ssp. lactis*
1	0	0	0	0	1	2 (1.3)	*Lb.salivarius*
4	3	4	2	4	4	21 (14.0)	*Lb. plantarum*
1	0	0	0	0	1	2 (1.3)	*Lb. cellobiosus*
0	2	4	2	6	2	16 (10.6)	*Lb. delbrueckii ssp. bulgaricus*
1	2	0	1	2	2	8 (5.3)	*Lb. helveticus*

Table 3. The most important morphological and biochemical properties of LAB isolated from "Uzicka" sausage

Count of isolates (%)	Identification (API 50CHL)	Cell morph.	CO_2 from glucose	Catalase	Growth at:					Slime formation
					4°C	10°C	15°C	37°C	45°C	
42(28.0)	***Lb.delbrueckii ssp. delbrueckii***	B	0	-	2(4.7)	30(71.4)	39(92.8)	42(100)	17(40.4)	0
21(14.0)	***Lb.plantarum***	B	0	-	9(42.8)	16(76.1)	20(95.2)	21(100)	0	0
16(10.6)	***Lb.delbrueckii ssp. bulgaricus***	B	0	-	0	7(43.7)	15(93.7)	16(100)	7(43.7)	0
15(10.0)	***Lb.fermentium***	B	0	-	6(40)	12(80)	13(86.6)	15(100)	2(13.3)	0
14(9.3)	***Lb.curvatus***	B	0	-	8(57.1)	13(92.8)	14(100)	14(100)	0	0
13(8.6)	***Lb.breviss***	B	0	-	5(38.4)	13(100)	13(100)	13(100)	5(38.4)	0
11(7.3)	***Lc.lactis ssp. lactis***	B	0	-	5(45.4)	10(90.9)	11(100)	11(100)	0	0
8(5.3)	***Lb.helveticus***	B	0	-	0	2(25)	4(50)	8(100)	6(75)	0
6(4.0)	***Ln.mesenteroides ssp.mesenteroides***	KB	3	-	2(33.3)	5(83.3)	6(100)	6(100)	0	6(100)
2(1.3)	***Lb.cellobiosus***	B	0	-	0	2(100)	2(100)	2(100)	0	0
2(1.3)	***Lb.salivariuss***	B	0	-	2(100)	2(100)	2(100)	2(100)	2(100)	0

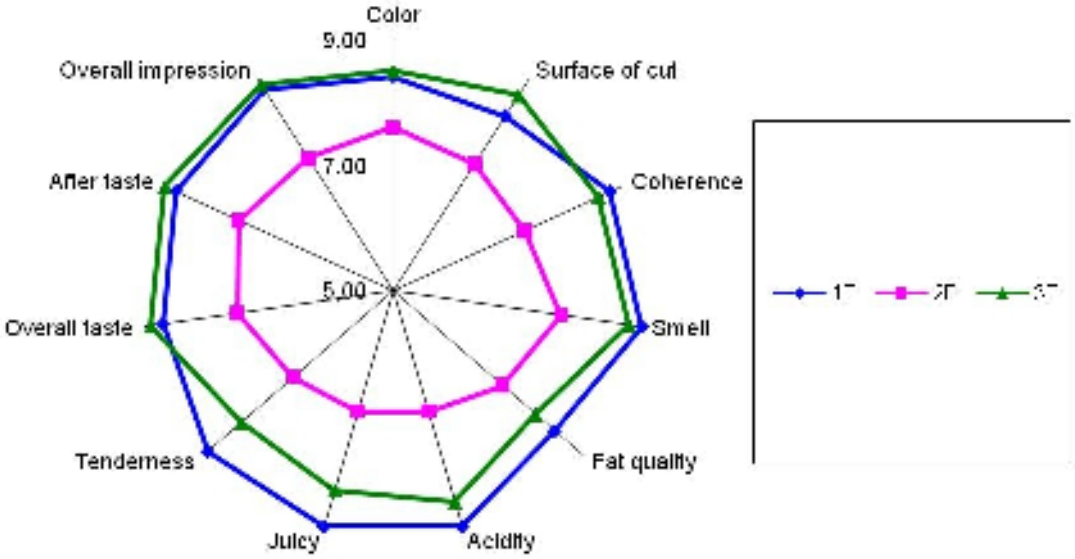
Color
9.00
Surface of cut
Coherence
Smell
Fat quality
Acidity
Juicy
Tenderness
Overall taste
After taste
Overall impression
7.00
5.00

Figure 1