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Production of biogenic amines by lactic acid bacteria isolated from Uzicka sausages

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Abstract

The aim of this study was to monitor production of seven biogenic amines (Cadaverine – CAD, Putrescine – PUT, Spermine – SPE, Spermidine – SPD, Histamine – HIS, Tyramine – TYR and Tryptamine – TRY) in selected 24 lactic acid bacteria (LAB) strains. The decarboxylase activity of the microorganisms was studied in growth medium after 24 h cultivation. The ability of 24 LAB isolates cultivated in MRS broth and M17 broth supplement with 0.5% glucose to produce biogenic amines was assessed using liquid chromatography tandem mass spectrometry (LC-MS/MS). The investigation showed that LAB isolated from Uzicka sausage are not significant producers of biogenic amines *in vitro*.

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1. Introduction

The number of meat product manufacturers that apply imported starter cultures in Serbia following modern trends is steadily increasing. As a rule, imported stater cultures are adapted to the needs of other markets. This results in products that lack traditional sensory properties which are most acceptable for the domestic consumers. Therefore, we started a series of investigations with the aim of selection of lactic acid bacteria (LAB) isolated from autochthonous fermented sausages. This will be the basis for the second stage – production of national starter cultures. Application of these cultures in manufacture of sausages would result in specific national products with characteristic and unique sensory properties which the Serbian population is accustomed to, and at the same time, it would be possible to improve quality parameters of such products[1].

Biogenic amines (BAs) are organic bases with aliphatic, aromatic or heterocyclic structures that primarily emerge by microbial decarboxylation of amino acids and can be found in a number of foodstuffs[2,3]. Many microbial species typical for fermented products inevitably lead to accumulation of biogenic amines, especially tyramine, 2-phenylmethylamine, tryptamine,

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cadaverine, putrescine and histamine. Intake of considerable amounts of BAs poses a health risk due to the effect of these compounds on gastrointestinal and nervous systems and the effect on blood pressure.

In general, BAs can be found in various foods and beverages such as fishery products, meat, dairy, vegetables, fruits, nuts, chocolate, wine, and beer[2,4]. Production of BAs in food depends on the presence of precursors, i.e. amino acids, as well as microorganisms that possess decarboxylation activity. In order for this process to be successful, favourable conditions are required for microbial growth and their enzymatic activities[5].

Many analytical techniques have been used for determination of BAs in the past decades, especially histamine having in mind its importance in scombroid poisoniong and regulatory requirements for its content in fish and fishery products. Thin layer chromatography being the first quantitative technique, has nowdays historical, rather than practical value. However, the majority of analytical methods for determination of BAs in various matrices are based on reversed-phase high performance liquid chromatography with either UV or fluorescence detection after pre-column or post-column derivatisation[5]. Liquid chromatography tandem mass spectrometry (LC-MS/MS) has been also employed in analysis of BAs recently, although availability of such instrumentation is still rather limited and cost of such analysis relatively high, especially for screening of large numbers of samples. However, high selectivity and sensitivity of LC-MS/MS makes this technique more than adequate for analysis of multiple BAs in various matrices.

1.1. Strains and growth conditions

At different stages of ripening of traditionally-fermented Uzicka sausages, during three repeated cycles of production, LAB were isolated using conventional microbiological techniques[7,8]. The LAB isolates from de Man-Rogosa-Sharpe (MRS) agar (Merck, KGaA, Darmstadt, Germany) were first checked by Gram staining and catalase reaction. Gram-positive and catalase-negative isolates were then identified by a commercially available biochemical identification system API 50CHL (bioMérieux, Marcy l´Etoile, France) and molecular methods[8].

LAB isolates (*Lactobacillus* and *Leuconostoc* spp.) were kept frozen at -20°C in de Man-Rogosa-Sharpe (MRS) broth (Merck, Germany) supplemented with 20% glycerol, while *Lactococcus* and *Enterococcus* spp were kept frozen at -20°C in Difco™ M17 Broth (BD Company, USA) supplemented with 0.5% glucose. Prior to use, the microorganisms were subcultured twice in 10 ml of MRS/M17 broth (1% inoculum, 24 h, 30°C). LAB isolates were then cultivated for 24 h in MRS/M17 broth, and 10 ml of liquid cultures were decanted in 10 ml polypropylene centrifuge tubes immediately after incubation. One half of the samples were analysed on the same day, while the other half was frozen and analysed on the next day after quick thawing in order to avoid subsequent formation of BAs. Both batches contained an aliquot of pure MRS/M17 broth in order to assess BA content in broth itself and prevent misinterpretation of results.

1.2. Biogenic amine production by LAB isolated from Uzicka sausage

Our analytical method for determination of BAs in MRS and M17 broth using LC-MS/MS is modified from the procedure originally proposed by Sagratini[9] for determination of biogenic amines in fish.

Analytical standards of BAs (histamine, cadaverine, putrescine, spermine, spermidine, tyramine, tryptamine) were purchased from Sigma-Aldrich (USA). Trichloroacetic acid (TCA) and ammonium acetate was obtained from J.T. Baker (The Netherlands). HPLC-grade acetonitrile (ACN), methanol (MeOH) and water was supplied by Sigma-Aldrich (USA). Ammonium hydroxide, ammonium acetate, formic acid and glacial acetic acid were purchased from Merck (Germany). Solid phase extraction (SPE) cartridges "Strata X 33 µm" polymeric sorbent were purchased from Phenomenex (USA). SPE manifold with 24 ports "Visiprep Standard Vacuum Manifold" was obtained from Sigma-Aldrich (USA

Aliquot of 5ml from each LAB strain culture was transferred using automatic pipette to the polypropylene centrifuge tube of 50 ml. A volume (15 ml) of 5% TCA was added and the mixture was homogenised for 1 minute at maximum speed using Bibby Scientific Ltd (UK) vortex, model SA-8. In order to prepare homogenate for SPE, pH value was adjusted to 11 using 25% ammonium hydroxide solution and indicator strips (Merck, Germany). SPE cartridges were conditioned with 4 ml of MeOH, followed by 4 ml of water using vacuum manifold system. Then, 4 ml of the sample were loaded onto the cartridges allowed to pass at flow rate of approx. 1 ml/min under gentle vacuum. Rinsing of cartridges was accomplished using 4 ml of MeOH/H_2O mixture (5:95, v/v) and cartridges were dried under vacuum in order to remove excess water. Graduated centrifuge tubes were placed into the manifold and BAs were eluted with 4 ml of the mixture MeOH/acetic acid (99:1, v/v). Eluted solution was evaporated under gentle stream of N_2 to dryness, reconstituted in 1 ml of 1% TCA, filtered into the autosampler vials and 10 µl was injected into the LC-MS/MS system.

LC-MS/MS analysis was carried out using Waters Acquity system with Waters TQD detector (Waters, USA). Separation was performed on Thermo Scientific Hypersil Gold (Thermo Scientific, USA), 100x2.1 mm, 3µm. Mobile phase was 10 mM ammonium acetate in 0.1% formic acid (mobile phase A) and ACN (mobile phase B) at flow rate of 0.3 ml/min. The gradient program was 0 min 20% B, 0-10 min 85% B, 15-25 min 20% B. Mass spectrometric analysis was performed in multiple reaction monitoring (MRM) mode. Positive electrospray (ESI+) was used for obtaining molecular ion. Temperature of the ionisation source and dessolvation gas (N2) were set at 120°C and 400°C respectively. Cone gas and dessolvation gas flow were 50 l/h and 550 l/h. Capillary voltage was 3500 V.

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