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Detection and identification of *S. carnosus* in starter cultures using real time PCR and subsequent HRM analysis of amplification products

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Abstract

Nowadays, the necessity to control species composition of commercial starter cultures and their growth during the ripening stage of fermented sausage in order to monitor the technological processes has became topical in Russia. However, there can be some difficulties in identification of an isolated culture when using biochemical methods. Using primers for amplification of bacterial species-specific regions makes it possible to identify starter cultures with a high degree of probability even without using TaqMan probes. Furthermore, using intraspecies polymorphism of the gyrase B subunit gene, we managed to subtype two *S. carnosus* isolates with the use of HRM analysis.

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Keywords: HRM analysis; species specific primers; starter cultures; *S. carnosus subsp utilis*.

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1. Introduction

World-wide accepted practice preferentially uses Generally Recognized As Safe strains of microorganisms in order to reduce microbiological risks when producing fermented food products[1]. However, microorganisms of the genus *Staphylococcus*, which are widely used in this sector, are not such. In this connection, the practice of substitution of *Kocuria varians* for microorganisms of the genus *Staphylococcus* as starter cultures has been introduced in Russia, which is reflected in the State Standard GOST R 55456-2013 "Dry sausages. Specifications." In connection with this, the necessity to control starter cultures and fermented semi-dry products manufactured with their use, for the presence of microorganisms of genus *Staphylococcus*, has emerged.

It is important to note that according to several authors, *S. xylosus* is a typical representative of the microbiota of traditional uncooked smoked sausages, while *S. carnosus* occurs far less frequently[2,4,5]. Thus, the presence of *S. carnosus* at a high level in a product can indirectly suggest that a starter culture containing this bacterium has been used.

2. Materials and methods

2.1. Selection of coagulase-negative cocci from starter cultures

The subjects of this research were 10 bacterial preparations, all of which contained *S. carnosus*. Control strains were *S. carnosus* Sta 1.1. (laboratory strain), *S. aureus* ATCC 25923, *S. epidermidis* CCM 2124. Detection of staphylococci was carried out by traditional microbiological methods: decimal dilutions according to ISO 6887-1, plating was performed on a surface of the Mannitol Salt Agar (MSA) (Liofilchem S.R.L., Italy).

2.2. Biochemical identification

Identification of the selected microorganisms was initially carried out with the biochemical test systems API Staph (BioMerieux France) and Microgen Staph ID (Microgen Bioproducts Ltd, UK).

2.3. DNA extraction

DNA extraction from the preparations and from a single pure colony was performed with the Sorb-GMO- B kit (Syntol, Russia) (Silica Based DNA Purification) according to the manufacturer's instructions.

2.4. Real-time PCR with subsequent melting curve analysis

Real-time PCR was performed with primer pairs developed by us, which were specific to the species *S. carnosus*, on the basis of the reference genome in GenBank: AM295250.1, locus_tag="SCA_2470", gene="gyrB"[6]. The reaction mixture with a volume of 25 µl included the primers with a concentration of 0.2 mM each, 10 µl of the reaction mixture with EVA Green (the final concentration per reaction: 2.5mM $MgCl_2$, 0.25mM dNTP, 2.5 E.A. SynTaq polymerase, 1x EVA Green) (Syntol, Russia) and 2 µl of extracted DNA.

Real-time PCR and melting curve analysis were carried out using an amplifier ANK-32 (Syntol, Russia).

PCR conditions included initial denaturation at 95°C – 420 s and 45 amplification cycles (60°C – 40 s, 95°C – 15 s), while melting curve analysis was performed at 0.1°C/step.

2.5. High resolution melting analysis (HRM)

HRM analysis was carried out on an amplifier LightCycler 96 (Roche, Germany) using LightCycler 480 HRM Master kit. HRM regime: 20 acquisitions /°C.