Screening strains for fermentation of meet raw material

Iryna Panasiuk[1], Svitlana Danylenko[1], Galyna Cherednichenko[2]

1 - The institute of food resources NAAS of Ukraine
2 - National University of Food Technology, Kyiv, Ukraine

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Corresponding author:

Iryna Panasiuk
E-mail:
Iruska_Pa@mail.ru

Abstract

Introduction. Promising technology in fermented meat products is the use of bacterial products containing the same composition in lactic acid bacteria and microorganisms of other taxonomic groups.

Materials and methods. Microbiological analysis of 6 samples of meat brine was conducted to study the quantitative and qualitative composition of microflora. Isolates were tested for Gram reaction, cell morphology, catalase formation, CO_2 production from glucose, hydrolysis of arginine, nitrate reduction, catalase and aroma forming activities, ability to grow at 10 °C and 45 °C and at pH 3 and 9.2, and for their tolerance to 4 % and 15% salt. Proteolytic activity of the cultures was determined at IPA medium with 5% NaCl and with 10% hydrolyzed milk.

Results. The number of bacteria in cm^3 of brine, does not exceed millions of cells. The most common genus in meat brine is Lactobacillus, Micrococcus, Staphylococcus. The presence of reduction of nitrate, catalase and aroma forming activities were discovered in 52 % of the selected strains of bacteria. The number strains capable of growth in present salt was decreased with the increasing salinity of the medium. Almost all crops grown in the temperature range (10-40) ^{0}C. The vast majority of strains of staphylococci was able to hydrolysis of milk proteins. Two highly productive staphylococci and five strains of lactic acid bacteria were selected by both biological and technological characteristics.

Conclusions. Properties of the selected strains of bacteria allows to attract the best of them for the manufacture of fermented meat products.

Introduction

Recently, the use of new, functional starter cultures with an industrially or nutritionally important functionality is being explored. Functional starter cultures offer an additional functionality compared to classical starter cultures and represent a way of improving and optimising the meat fermentation process and achieving tastier, safer, and healthier products. Examples include microorganisms that generate aroma compounds, health-promoting molecules, bacteriocins or other antimicrobials, contribute to cured meat colour, possess probiotic qualities, or lack negative properties such as the production of biogenic amines and toxic compounds. The vast quantity of artisan fermented sausages from different origins represents a treasure chest of biodiversity that can be exploited to create such functional starter cultures.

In the production of cured meat products staphylococci and lactic acid bacteria (LAB) are the most important bacteria apply to the meat products.

Lactic acid bacteria play an essential role in the production of fermented meat products, *Lactobacillus* being the main species used in the European type of fermented products. In order to ensure sensory quality and good color formation, lactic acid bacteria are not sufficient and the contribution of *Staphylococcus carnosus* is needed. Some strains of meat lactobacilli exhibit important properties from a technological point of view, such as the production of antimicrobials. The application of bacteriocinogenic lactic strains as starter cultures in fermented products could provide an additional tool for preventing the outgrowth of food pathogens in sausages as well as enhancing the competitiveness of the starter organisms in favor of the fortuitous flora. However, further research is needed before the application in meat products of these bacteriocins can be put into practice. The spectrum of application of lactic acid bacteria is wide; lactobacilli are good candidates as probiotic strains, thanks to their GRAS status and their ability to adhere to epithelian cells. In the near future, lactobacilli probiotic cultures will be included in new foods; fermented meat products are likely to be one of these foods.

The production of organic acids - mainly lactic acid - from carbohydrates is the major role of LAB in sausage fermentation. This depends on several chemical, physical and microbiological reactions. While acidifying the batter, LAB participate in the coagulation of muscle proteins, resulting in the increased slice stability, firmness and cohesiveness of the final product They also enhance the spontaneous reduction of nitrites to nitric oxide, which reacts with the myoglobin to form nitritmyoglobin, the compound responsible for the typical pink color of cured sausage. Moreover, they contribute to the flavor of the final product through the formation of noticeable acidic and vinegary (acetic acid) tastes. Acidic conditions are also thought to increase the activity of cathepsin D, which is responsible for muscle proteolysis. The production of organic acids is undoubtedly the determining factor on which the shelf life and the safety of the final product depends. The inhibition of pathogenic and spoilage flora is also dependent on a rapid and adequate formation of these organic acids. Finally, it has been reported that a rapid decrease in pH caused by amine-negative starter cultures can largely prevent biogenic amine (BA) accumulation in sausages.

The critical features for selected staphylococci are pH tolerance and temperature tolerance enabling them to produce important enzymes under the relatively low temperature conditions present during fermentation. Staphylococci enhance color formation and color stability in addition to repressing hydrogen peroxide induced rancidity. The most commonly applied staphylococci are *Staphylococcus carnosus* and *Staphylococcus xylosus*, beneficially in a blend, as both strains produce different relevant functional components.

Together with proteolytic and lipolytic enzymes in the meat the enzymes produced by staphylococci contribute also to the aroma formation.

Besides, the most promising starter strains are those isolated from naturally fermented meat products once they are the dominant and the well adapted population.

The aim of this work the brine microflora, used in the manufacture of meat products was investigation and search for strains of different taxonomic groups with high levels of biochemical activity.

Materials and methods

6 brines samples were taken for microbiological examination. These brines within pH 6.4 to 6.6 was used for various raw meat. Samples of brine were selected for different duration of pickles. Brines for № 1-3 ham "special" was taken after 7 days of pickles, pickles № 4-5 for ham - 2 days.

One milliliter of each sample homogenate was diluted serially tenfold in saline solution (0.85% NaCl). Diluents (0.1 ml) were plated on appropriate agar medium for microbiological analysis.

A total of 57 isolates were identifed by comparing the morphological, physiological, and biochemical characteristics of the strains. Isolates were tested for Gram reaction, catalase formation, cell morphology, CO_2 production from glucose, hydrolysis of arginine, nitrate reduction, catalase and aroma forming activities.

They were also tested for their ability to grow at 10 °C and 45 °C and at pH 3 and 9.2, and for their tolerance to 4 % and 15% salt. Proteolytic activity of the cultures was determined at IPA medium with 5% NaCl and with 10% hydrolyzed milk.

Growth in high salt concentration was observed after 3 days of incubation at 37 °C on MRS agar (Merck, Darmstadt, Germany) plates added with 3.0 and 9.2% of NaCl (Merck, Darmstadt, Germany), respectively. Growth in the presence of commercial curing salt was observed after 3 days of incubation at 37 °C on MRS agar (Merck, Darmstadt, Germany) plates added with commercial curing salt (Cura 102 - Duas Rodas Industrial Ltda, Jaraguá do Sul, Brazil), with sodium nitrate and sodium nitrite in respective concentrations of 300 and 150 $mg.kg^{-1}$.

For the nitrate reductase test, a swab of each culture on selective agar (Merck, Darmstadt, Germany) plates (anaerobically incubated at 37 °C for 48 hours) and suspended in sterile peptone water 0.1% with turbidity equivalent to 0.5 McFarland. A 1.0 ml aliquot of homogenized bacterial suspension was added to a sterile tube containing nitrate broth (DIFCO, Lawrence, USA). All tubes were incubated anaerobically at 37 °C for 48 hours. After the incubation period, 1 drop of each reagent of the NIT test (NIT 1 + NIT 2 reagents bioMérieux®sa, Marcy l'Etoile, France) was added to each tube. After 10 minutes, the presence of red color indicated positive reaction to the reduction of nitrate to nitrite. A negative control with no substrate and a positive control with a culture of *S. xylosus* positive for nitrate redutase were used.

Belonging Gram positive cocci and catalase positive cocci to the genus Staphylococcus installed in the following diagnostic tests : fermentation of glucose to form acid under anaerobic conditions; capacity for oxidation of glycerol in the presence of erythromycin (0.4 mg/l); sensitivity furazolidon (disks 100 mg); resistance to lysozyme.

Analysis confirmed the purity of the culture of staphylococci and partitioned mixed culture if necessary.with rapid diagnostic system «Diastaf». Preparation: using a disc with an antibiotic batumin can reliably and quickly (within 18 hours). Differentiate Staphylococcus area for growth inhibition around the disc from other Gram-positive cocci

that are insensitive to the drug. Preparation " Diastaf " does not affect the growth of microorganisms other taxa and provides rapid diagnosis of staphylococci in mixed cultures The drug is intended for the detection of staphylococci in clinical , veterinary and research institutions.

The presence of coagulase define a preliminary assessment of the degree of safety of staphylococci was performed according to ISO IDF 138:2003. For positive and negative controls were reacted with typical collector strains of S. aureus HISK 049,065 and Kocuria varians ATCC 9341 , respectively.

Results and discussion

Lactic acid bacteria predominated particularly (35-43%) in brine for ham, whereas advantage on the side of coccoid forms - micrococci and staphylococci (33-36%) was in brine for balyk. A significant proportion were yeast (11-17%) and spore-forming bacteria - from 17 to 24% (Tab.1). Content of sanitary exponential mikrofalora didn't exceed 11%.

27 isolates catalase positive cocci was isolated from brine applying the diagnostic tests, and 20 of them (74.4%) assigned to the genus Staphylococcus.

In previous assessments of the safety 20 strains of staphylococci was isolated, of them the 3 strains (15.2%) was found that catalase positive cocci and potentially dangerous. They were removed from further work.

This isolated strains (82%) were able to reduction nitrate / nitrite and the rest strains didn't have this property.

Almost all crops grown in the temperature range (10-40) ^{0}C. The number strains capable of growth in present salt was decreased with the increasing salinity of the medium. Thus respectively for 100% and 96% of strains growth recorded if the concentration of sodium chloride 4.0% and 6.5%, whereas only 80% strains increased in content 10% of NaCl.

pH is a major factor limiting the growth of staphylococci, they was active in acidity pH of 4.5 units of the medium. In this acidity only 64% were viable of the investigated strains.

Taste and aroma of damp-dried products produced by lipolytic and proteolytic activity of microorganisms. Meat proteins are broken down into free amino acids under the action of the proteolytic activity of bacterial cultures that are directly involved in the formation of taste.

Thanks lipolytic activity of microorganisms volatile fatty acids was produced, which are further converted to carbonyl compounds contribute to the formation and flavor of the finished product. The consistency of meat products also depends on the muscle proteins (sarcoplasmic and miofibrylyarnyh). The stronger proteolysis occurs in meat, the more tender it becomes relevant role in this process is played by bacterial culture that affect consistency due to its proteolytic activity.

Research by strains of proteolytic activity showed that the vast majority of strains of staphylococci was able to hydrolysis of milk proteins. Thus, of the 17 strains studied 3 - formed enlightenment zone diameter of 10 cm, 6 - 13 cm to 15 cm, 2 - 20 cm for the remaining 6 proteolytic activity was not observed. The largest diameter of the zone of enlightenment characterized S. xylosus 5307 - 24 cm.

Table 1

Brine microflora

Sample	The total number of microorganisms,UFM/g	Value for groups of microorganisms, %				
		LAB	*MK+ST*	*YE*	*CF*	SIM
Brine 1 for ham,	$1,4 \cdot 10^6$	43	10	16	22	9
Brine 2 for ham,	$4,2 \cdot 10^6$	38	15	17	24	6
Brine 3 for ham,	$5,6 \cdot 10^6$	35	26	11	18	10
Brine 4 for balyk	$1,3 \cdot 10^6$	25	35	14	17	9
Brine 5 for balyk	$1,3 \cdot 10^6$	20	33	15	21	11

Footnotea. *LAB* - lactic acid bacteria; *MK+ST* - micrococci and staphylococci; *YE* - yeast, *CF* - spore-forming bacteria; *SIM* - sanitary indicative microflora.

Conclusions

The qualitative and quantitative composition of microflora meat brine was studed. Five samples of industrial brine microflora have been explored. It was determined that the number of bacteria in 1 cm^3 reaches 10^6 CFU, the main part is a genera Lactobacillus, Micrococcus, Staphylococcus. The most common genera in brine, used for preserving for meat Lactobacillus, Micrococcus and Staphylococcus. Two highly productive staphylococci and five strains of lactic acid bacteria were selected by both biological and technological characteristics. The presence of nitrate reducing, catalase and aroma forming activities were discovered in selected strains of bacteria, so they are promising for the manufacture of fermented meat products.

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