EFFICIENT ACTIVITY OF COLLAGENOLYTIC MICROBIAL ENZYMES AT LOW POSITIVE TEMPERATURES

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The international experience and modern trends in the development of meat processing point out the potential use of biologically active substances for the creation of products with predetermined characteristics.
One of the most important factors is their ability to render effect on the connective tissue of meat raw material. In this connection first and foremost is the search of enzymes which would break down the collagen.
Some studies were published (1, 3, 4, 5) concerning the isolation of collagenolytic enzymes from various sources: vertebrates, insects, crustaceans, plants, and microorganisms as well. However, little information about enzymes which could break down collagenic fibres at low temperatures is available till now. Some of the mentioned substances appeared to be rather exotic, particularly those isolated from crab Uca pugilator hepatopancreas .

PURPOSE

The purpose of the study was to search for some producers and collagenolytic enzymes of microbial origin acting efficiently at low positive temperatures (from 0 to 6 ^{0}C).
Specific stamms with low proteolytic activity and high collagenolytic activity were isolated by means of the screening process.

OBJECT AND METHODS

As an object of investigation, meat (beef and pork), connective tissue, mincemeat, brines, leather raw material, and museum microorganism cultures were used.
In the course of fulfillment of the present work methods given below were used. The primary screening of natural microorganisms for revealing of perspective stamms-producers was carried out on dense nutrient media, namely, on beef-extract gelatin (BEG) and L-broth with 20 per cent content of gelatin. For identification of microorganisms producing proteolytic enzymes with wide substrate specificity milk agar was used. By formation of casein hydrolysis zone judged about production of non-specific proteinases by them. The quantitative estimate of proteolytic and collagenase activity of the cultures under investigation was carried out using azo casein and azo coll as non-specific substrate. The measurements were made using device MULTISCAN (Bio-Rad, USA).
From all investigated stamms were selected those which were characterized by availability of maximum gelatinolytic and low proteolytic activity, and their ability to break down native collagen at low temperatures (from 4 to 6 °C) was studied. Concentration of protein in enzyme samples was determined by the modified Bredford method. For the unit of activity by collagen was taken hydrolysis of 1 µM of substrate during 1 min per 1 mg of protein in the enzyme preparation.
Distribution of the enzyme between the culture liquid and biomass of microbe cells was studied. Accumulation and activity of the enzyme was determined by hydrolysis of the native collagen (Acid Soluble From Rat Tail - Type I) and on meat raw material.

RESULTS

Analysis of psychrophilous museum and natural stamms was carried out. The bulk of museum cultures was constituted by different stamms of Pseudomonas genus. Higher attention to this genus of microorganisms was explained by their ability to efficient growth at low positive temperatures and availability of secretory collagenolytic enzymes in some characterized stamms. The rest of the cultures were classified with Bacillus genus.
The results of investigations of natural sources of collagenolytic microflora are given below.
From meat (beef and pork without large inclusions, connective tissue) were isolated 124 stamms-producers, from the connective tissue - 35, brines - 23, leather raw material - 22. Thus, from natural sources were revealed 204 stamms-producers of gelatinolytic enzymes that actively break down BEG. Museum cultures had lower activity.
As a result of testing of revealed stamms-producers on gelatin layer, the highest activity had 15 from 204 investigated stamms-producers. Availability of activity was determined by the degree of hydrolysis of gelatin layer. During cultivation of the above cultures on milk agar, all of them formed caseine hydrolysis zones, what pointed to secretion of non-specific proteinases by them.
Moreover, size of zones for variable stamms differed significantly, however, one of them was characterized by availability of maximum gelatinolytic and low total proteolytic activity. Investigations of the stamm for pathogenicity showed the negative result.
The further tests showed its high collagenolytic activity, as compared with the other stamms.
These properties didn't change even after lyophilic dehydration of the culture.
Collagenase activity of the reduced dry bacterial preparation, six-hour broth culture, and three- and five-day culture liquid was assessed on meat substrates in laboratory conditions. The water-binding capacity and plasticity of meat were the basis of assessment.

Improvement of the above properties under the influence of enzymes of the tested cultures was determined..

CONCLUSION

Stamms well cultivated at low positive temperatures and producing collagenolytic exoenzymes were isolated. They have low total proteolytic and sufficient collagenolytic activity. When influencing meat raw material, they improve its qualitative characteristics.

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