MORPHOLOGISCHE UND BIOCHEMISCHE UNTERSUCHUNGEN EINIGER VON FLEISCHERZEUGNISSEN ISOLIERTEN HEFESTÄMME

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Zusammenfassung

Es wurden 67 Hefestämme von Rohwürsten isoliert, von welchen zwei Stämme - K_1 und K_{12} mit hohen Aktivitäten der Nitratreduktase, der Katalase, der Peroxydase und anderer Enzyme ausgewählt wurden.

Die untersuchten Stämme zeigen eine gute Entwicklung in festen und flüssigen Nährböden und in einer Fleisvh-Pepton-Bouillon mit einer Zugabe von 5% Natriumchlorid. Man beobachtet dabei eine gute Zuckerfermentation, ohne Gasbildung, und eine Assimilation verschiedener Kohlenhydrat- und Stickstoffquellen.

Die besser ausgeprägte Nitrat-Reduktase-Aktivität des Stammes K_{12}, die von einem hohen Niveau der Katalase- und Peroxydase-aktivität in der exponentiellen und stationären Entwicklungsphase begleitet ist, stellt einen Nachweis für ihre Rolle bei der Bildung und Erhaltung der Farbe bei Dauerwürsten während ihrer Reifung dar.

Diese biochemischen Eigenschaften und Verhalten des Stammes K_{12} beweisen seine aussichtsreiche Anwendung als Starterkultur bei der Herstellung von rohgetrockneten Fleischprodukten.

ETUDES MORPHOLOGIQUES ET BIOCHIMIQUES SUR CERTAINES SOUCHES - LEVURES, ISOLEES DE PRODUITS CARNES

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Résumé

On a isolé 67 souches - levures des saucissons secs. On en a séléctionné deux souches - K_1 et K_{12} possédant une activité enzymatique, nitrate-réductasique, catalasique, peroxydasique, etc. élevée.

Les souches étudiées se développent bien sur les milieux nutritifs solodes et liquides, ainsi que dans un bouillon peptoné de viande, additionné à +5 % du chlorure de sodium. Elles fermentent facilement les sucres, sans formation de gaz, et assimilent les différentes sources d'azote et d'hydrates de carbone.

L'activité nitrate-réductasique, mieux exprimée dans la souche K_{12}, accompagnée d'un niveau élevé d'activité catalasique et peroxydasique dans les phases exponentielle et stationnaire du développement, prouve le rôle qu'elle a pour la formation et la préservation de la couleur pendant la maturation des saucissons secs.

Ces caractéristiques biochimiques de la souche K_{12} prouvent l'effectivité qu'elle peut avoir comme culture-starter pour la fabrication des produits carnés tels que les saucissons secs.

MORPHOLOGICAL AND BIOCHEMICAL INVESTIGATIONS ON ISOLATED FROM MEAT PRODUCTS YEASTS STRAINS

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Summary

From raw dry sausages were isolated 67 yeasts strains. From them are selected two strains - K_1 and K_{12}, which exibit high nitrate-reductase, catalase, peroxydase and other enzymatic activities.

The investigated strains grow well on hard and liquid media and in MPB + 5 % sodium chloride. They ferment sugars without gas formation and utilize different carbohydrate and nitrogen sources.

The better exibited nitrate-reductase activity of strain K_{12} along with a high level of catalase and peroxydase activity in the exponential and stationary phase of growth, prove its role in the formation and colour keeping of raw dried sausages during their ripening.

These biochemical properties and behaviour of strain K_{12} make it perspective for use as a strater culture in the production of raw dried meat products.

МОРФОЛОГИЧЕСКИЕ И БИОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ НЕКОТОРЫХ ШТАММОВ ДРОЖЖЕЙ, ВЫДЕЛЕННЫХ ИЗ МЯСНЫХ ПРОДУКТОВ

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Аннотация

Из сыровяленых колбас выделены 67 штаммов дрожжей. Из них отобраны два штамма - K_1 и K_{12} с высокой нитратредуктазной, каталазной, пероксидазной и др. энзимными активностями.

Исследованные штаммы хорошо растут и развиваются на твердых и жидких питательных средах и на МПБ + 5% NaCl . Ферментируют сахара, без образования газа и усваивают различные углеводные и азотные источники.

Более выраженная нитратредуктазная активность штамма K_{12} и высокий уровень его каталазной и пероксидазной активности в экспоненциальной и стационарной фазах развития доказывают его положительную роль при формировании и сохранении цвета сыровяленых колбас во время их созревания /сушки/.

Эти биохимические свойства штамма K_{12} доказывают его перспективность для использования в качестве стартерной культуры при производстве сыровяленых продуктов.

MORPHOLOGICAL AND BIOCHEMICAL INVESTIGATIONS ON ISOLATED FROM MEAT PRODUCTS YEASTS STRAINS

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Ripening of raw dried sausages is a complex microbiological and biochemical process in which a definite role is plaid by different kinds of microorganisms - lactic acid bacteria, micrococci, moulds, yeasts and others.

In our present work we want to investigate the morphological and biochemical properties of two strains of yeasts in connection with their use as starter cultures in the production of raw-dried sausages.

In this connection exist quite opposite opinions about the role of yeasts in this process. Some authors (4,6,7,10,11) assign a major role to yeasts in the ripening of raw-dried sausages, taking part in the formation of the specific ta ste and flavour, from the changes in fats and proteins. Other authors (1,3,8) are treating them as invoking unwanted changes in the organoleptical properties of the product.

Material and Methodics

The isolated in our laboratory two strains of yeaste are selected after biochemical tests as most convenient among all 67 strains isolated from raw-dried sausages.

For deciding their culturemedium characteristics was used liquid nutritive medium - Ma lz broth of 6°Bl. Marked was the property to form ring, turbidity and sediment during 3 to 10 days. For the morphological characteristics - form, size, structure and appearence of the cells, was used malz gelatine. Determination of form, size and type of colony was made by inoculation of very thin 24-hours culture on malz-agar. The petri are kept under room temperature in exica tor for 30 days.

For determination of the biochemical and physiological properties of the strains are made tests for: behavior to non nitrogenous sources of carbon/carbohydrates, organic acids, alcohols/, gaz formation, growth in the presence of 5% NaCl, proteolytic activity, proving of nitrogen-reductase activity, quantitative determination of catalase and peroxydase activities.

The behavior of the yeasts strains to non-nitrogenous sources of carbon was proved by two methods: by fermentation of carbohydrates and by oxydation. Fermentation was determined by the Durham fermentation tubes with the following 1% sugar solutions: glucose, fructose, galactose, sa ccharose, la ctose, maltose, inoulin.

T he use of these sugars by oxida tion was proved following the auxonographic method a fter Beijerinku, modified after Loder, using the cultures of Odintzova a nd Rieder. Inoculations were cultivated at 26°C for 24-48 h. For the property of the yeasts strains to use a given sugar is decided by their growth on hard culture containing the tested carbon containing source.

The proteolytic activity of the strains was proved by needle inoculation in malz gelatine, while for their nitrate-reductase activity was used MPB + 1% sodium nitrate and then reagent of Gries. The reductase activity was determined to milk with 1% methylene blue. For the property of the strains to grow with 5% NaCl was used MPB + 5% NaCl. Spore formation on culture after Gorodkova, and fats denaturation on culture of sterile beef tallow with a very thin layer of agar with 1% $CaCO_3$. After inoculation and incubatio n for 48 h a t 26°C, around the colonies denaturating the fats is obtained a clearly seen transparant zone.

T he catalase activity we determined quantitatively after the modified method of Krajnev (1962), and the peroxydase activity after the modified method of Popov (1971). Material for investigation was taken from the exponential phase (10th hour after inoculation) and the stationary phase of growth (24th hour). The investigations were made with washed reposing cells in culture medium. The enzyme activity is espressed in units calculated on 100 ml culture medium or 100 mg cell carbon. The results from the biochemical investiga tions are statistically proved for significance after the variations method (Sepetliev D., 1968).

Results and Discussions

The morphological, cultural and biochemical properties of the investigated yeasts strains are given in 5 tables.

Table 1

Morphological characteristics

Strain	Type and size of cells	Growth in MB	Type and size of colony	Spore formation	Pseudomicell formation
K_1	Round, oval or single or pairs 3-5μx 4-8μ	Thin layer ring and fl.sediment	Round, sleek yellowish white, pleasent odour 28/28 mm	-	-
K_{12}	Elongated 3-5μx 5-9μ	Layer, ring fl.sediment	Round, greyish yellow piled center pleasent odour 25/25 mm	-	Well expressed

As seen from the table, the differences betwen the two strains are expressed in the form, size of the cells, colour of colonies and most of all after the well defined pseudomicell of strain K_{12}.

Table 2

Sugar fermentation

Strain	Glucose	Fructose	Galactose	Saccharose	Maltose	Lactase	Inulin	Rafinose
K_1	+++	+++	+++	+++	+++	±	+	++
K_{12}	+++	+++	+	+++	+++	-	-	+

From the table is quite clear that the investigated strains of yeast have an well expressed fermentation ability. To a lesser extend are fermented galactose and rafinase by strain K_{12} and lactose from both strain. A characteristic property for both strains is that in fermentation there is no gaz formation.

Table 3

Use of nonnitrogen and nitrogen sources

Strain	Carbohydrates: Glucose	Galactose	Xilose	Ethanol	Peptone	Nitrogen sources: $(NH_4)_2SO_4$	KNO_3	$NaNO_3$	$NaNO_2$
K_1	+++	++	+	+++	+++	+++	+++	+	+
K_{12}	++	++	+	+++	+++	+++	++	++	+

From the results above is seen that the investgated strains are well using carbohydrates as well as nitrogen sources. K_{12} in comparison with K_1 makes less use of glucose and KNO_3 and better use of $NaNO_2$.

Table 4

Development and biochemical activity

Strain	Devel. in MPB + 5 % NaCl	Denat. of fats	Milk reduction with 1 % meth.blue	Proteolitic activity	Nitrogen reduction activity
K_1	+++	+	+++	+++	++
K_{12}	+++	+	+++	+++	+++

From the table it is clear that the strain K_{12} exibits a stronger nitrate reductase activity in comparison with the same of strain K_1.

Table 5

Catalase and peroxydase activity during different periods of development

Strain	Exponential phase: on 100 ml cultural liquid	on 100 ml cellular carbon	Stationary phase: on 100 ml cultural liquid	on 100 ml cellular carbon
	Catalase activity			
K_1	0,67 ± 0,015	0,842 ± 0,020	0,853 ± 0,015	1,058 ± 0,020
K_{12}	0,527 ± 0,005	0,681 ± 0,007	0,726 ± 0,010	0,859 ± 0,016

	Peroxydase activity			
K_1	83,5 ± 8,2	374,2 ± 15,6	103,4 ± 7,4	460,3 ± 12,8
K_{12}	69,1 ± 5,7	263,5 ± 9,0	81,0 ± 6,5	346,7 ± 10,3

The value expressed on table 5, show that both strains have a high level of catalase and peroxydase activity. The catalase and peroxydase activity of strain K_1 is higher from the on of strain K_{12}. The enzymatic activity of both strains depends on the age of cells and is significally higher in the stationary phase of development. It is proved that the catalase and peroxydase activity of the microbial cells of strains K_1 and K_{12} are significantly higher than the one of the cultural liquid.

Discussion

The characteristics of both strains prove their high biochemical activity which determines their role in the ripening of the products.

The better expressed nitrate-reductase activity of strain K_{12} and its higher level of catalase and peroxydase activity in both phases of exponential growth, probe its positive role in the formation and colour-keeping of raw-dried sausages during their ripening. These indices, compared with the same from certain investigated micrococci and lactic acid bacteria used, as starter cultures, are manyfold higher. This shows their perspectivity as starter cultures in the production and ripening of meat products.

Literature

1. Adamović N., A.H.Beganović, F.Hadžihalilović, Z.Foršek. Prilog poznavanju vrsta funga u nekim polutrajnim kobasicama i suhomesnatim proizvodima. Tehnologija mesa, 1972, 5, 130-132.
2. Sepetliev D. Statisticheski metodi za obrabotka na danni ot medizinski nauchni prouchvania. Medizina i fizkultura, 1968,Sofia
3. Francetić M., M.Haajsig, J.Šimac. Gljivična flora Gavrilovićeve salame tijekom zrenja. Tehnologija mesa, 1971, 10, 298-302.
4. Kondereški S., J.Đorđević, J.Jarševac. Prilog nalázu kvasaca na mesu u salamuri i mesnim proizvodima i njiha uticaj na miris i urus gotovih produkata. Tehnologija mesa, 1972, 3, 66-72.
5. Krainev S.I. Catalaznaia activnost. Biohimia, 27, 1962, 5, 79
6. Lensen L.B. Microbiology of meat, 1945.
7. Leistner L., J.Ayers, D.A.Lillard. IVth Symposium of the world assotiayion of veterinar food hygienists, Lincoln, 1965.
8. Nickerson J., A.Sinskey. Microbiology of food processing, 197
9. Popov T., L.Neikovska. Method opredelenia peroxydaznoi activnosti krovi. Gigiena i sanitaria, 1971, 10, 44.
10. Zlatev I., M.Litvinenko. Prouchvane varhu bialoto oblojenie na loukankite. Nauchni trudove NITPKIM, vol. III, 1965, Sofia.
11. Zlatev I., D.Milev. Microbiologia i microbiologichen kontrol na hranitelnite produkti, 1969, Sofia.