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STUDY OF CULTURAL AND BIOCHEMICAL PROPERTIES OF *PENICILLIUM CANDIDUM* STRAIN

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ABSTRACT

The article presents the results of the study of morphological, cultural, and biochemical properties of the *Penicillium candidum* strain isolated from salami. The strain was cultivated on various nutrient media to assess the nature of its growth and the structure of the mycelium. On solid media, differences in the color, shape, and surface of colonies were observed. The best growth was noted on potato and honey agars, where colonies exhibited radial furrows and a wrinkled surface. In liquid media, dense mycelial globules formed in the absence of surface and wall-associated growth. Biochemical studies showed that *Penicillium candidum* degrades glucose and maltose, weakly ferments lactose, and exhibits urease activity. In antagonistic tests, the strain demonstrated the ability to inhibit the growth of *Bacillus* spp., forming a growth inhibition zone with a diameter of 24 mm. The concentration of the antibiotic substance was 10.6 µg/ml. The *Penicillium candidum* strain demonstrates high biochemical activity and antagonistic properties, confirming its potential for application in the food industry as a biopreservative or technological culture.

Key words: *nutrient media, Penicillium candidum, morphological properties, cultural properties, antagonistic properties.*

1 INTRODUCTION

Fungi of the genus *Penicillium* are an essential part of microbiological and biotechnological research [1–5]. They attract attention due to their ability to produce diverse metabolites, including antibiotics, enzymes, and organic acids [6, 7]. Among the representatives of this genus, *Penicillium candidum* (synonym: *Penicillium camemberti*) holds a special place, being widely used in the food industry, particularly in the production of white-mold cheeses such as Camembert and Brie [8]. The importance of studying the cultural and biochemical properties of this microorganism lies in its key role in shaping the organoleptic characteristics of products [9], as well as its potential for use in other biotechnological processes [10, 11].

The scientific study of *Penicillium candidum* opens up many opportunities for improving food production technologies and developing new biotechnological solutions [12]. This fungus participates in the hydrolysis of proteins and lipids, contributing to the formation of flavor and aromatic compounds [13]. Additionally, the enzymes produced by *P. candidum* may be beneficial in the pharmaceutical and chemical industries. Nevertheless, the strains of this fungus exhibit high biochemical and cultural variability, requiring detailed research into their properties and mechanisms of action [14, 15].

The modern food industry focuses on improving product quality, promoting sustainable production, and reducing losses. In this context, understanding the cultural and biochemical properties of *P. candidum* enables the creation of new strains with desired characteristics or optimization of cultivation conditions [16]. Moreover, the need to study the safety of using this microorganism and its possible interactions with other microbial communities emphasizes the relevance of this topic. In the context of global market expansion and increased competition in the cheese industry, studies of *P. candidum* are of particular importance [17].

Research on *P. candidum* is actively conducted in various directions. Scientists study the optimal growth conditions of the fungus, including temperature, humidity, medium acidity, and nutrient content [18]. Special attention is given to the enzymatic activity of *P. candidum*, particularly lipases, proteases, and catalases [19]. Thanks to advances in genomics, scientists have deciphered the genome of *P. candidum*, allowing the identification of genes responsible for the synthesis of key metabolites [20]. Toxicological studies have shown that *P. candidum* strains used in the food industry are safe for humans [21, 22].

The purpose of this study is to investigate the cultural and biochemical properties of *P. candidum*, a promising direction that combines the interests of microbiology, biotechnology, and the food industry.

2 MATERIALS AND METHODS

The material for the study was the *P. candidum* strain isolated from a finished salami product. The following nutrient media were used in the study:

Czapek agar containing: sucrose – 30 g, agar – 15 g, sodium citrate – 2 g, potassium phosphate – 1 g, magnesium sulfate – 0.5 g, potassium chloride – 0.5 g, iron sulfate – 0.01 g;

Honey agar (per 100 ml): honey – 8 g, peptone – 2 g, agar – 1.8 g;

Potato agar (4.9 g of powder per 100 ml of distilled water);

Corn agar: 4 g glucose, 1 g NaCl, 4 g corn flour, 1.8 g agar per 100 ml of distilled water;

Sabouraud agar: glucose – 4 g, peptone – 2 g, NaCl – 1.8 g, agar – 4 g;

Sabouraud broth: enzymatic dry peptone – 12.0 g, yeast extract – 5.0 g, NaCl – 5.0 g, D-glucose – 30.5 g.

Giss medium (glucose, sucrose, maltose, mannitol, lac-

tose);

Christensen's medium with 40% urea.

Research methods: microscopic, biochemical, and cultural.

Preparation of nutrient media Sabouraud dextrose agar was prepared according to the composition listed above. The resulting mixture was boiled for 1 minute to completely dissolve the components and then sterilized by autoclaving at 0.5 atm (110°C) for 15 minutes. Before pouring the medium, it was thoroughly mixed and cooled to room temperature.

Preparation of Sabouraud broth to prepare Sabouraud broth, 10 g of the nutrient mixture powder was stirred with 100 ml of distilled water. The mixture was boiled for 1 minute and sterilized at 121°C for 15 minutes. After sterilization, flasks were inoculated with the fungal culture and cultivated for 6 days until globules were formed.

Inoculation technique for nutrient media. Inoculation on solid media (honey, corn, Czapek, Sabouraud, and potato media) was carried out under sterile conditions in a laminar flow cabinet after pre-sterilization of the workspace. Inoculation was performed by touch or stab method. Petri dishes were labeled with the material name, culture number, and inoculation date. Plates with inoculations were kept in a thermostat under appropriate conditions for no more than 5 days.

Submerged cultivation in Sabouraud broth for submerged cultivation, fragments of colonies formed on solid media were used. In each liquid medium, 0.1 cm² of inoculum culture was added per 100 cm² of medium volume.

Study of biochemical properties of *P. candidum* to study the saccharolytic activity of *P. candidum*, Hiss media with different carbohydrates (sucrose, glucose, lactose, maltose, and mannitol) were used. Hiss medium was prepared by dissolving 0.75 g of powder in 50 ml of distilled water, boiling for 3 minutes until completely dissolved, and then sterilizing by autoclaving. To test urease activity, Christensen's medium containing 5% urea was used.

Study of antagonistic properties. Preparation of potato-glucose agar was carried out according to the formulation and poured into Petri dishes. Approximately 1 gram of *P. candidum* culture was inoculated in the center of the dish, pre-inoculated with *Bacillus* spp. Observations were conducted for three days.

3 RESULTS

The *P. candidum* strain, isolated from a ready-to-eat sausage product (salami), was cultivated for 5 days at a temperature of 28°C on solid nutrient medium – Sabouraud agar. The growth of the culture was observed over a period of 15 days. The isolation of the pure *P. candidum* strain is shown in Figure 1.

As shown in Figure 1B, the colony has a white color with a velvety surface, a raised center, smooth edges, and a dense consistency. The reverse side of the colony is light yellow.

Microscopy of the fungus was performed using a light microscope at 40× magnification. The sample was examined in detail under an immersion system at 100× magnification (Figure 2).

As shown in Figure 2, the *Penicillium candidum* sample

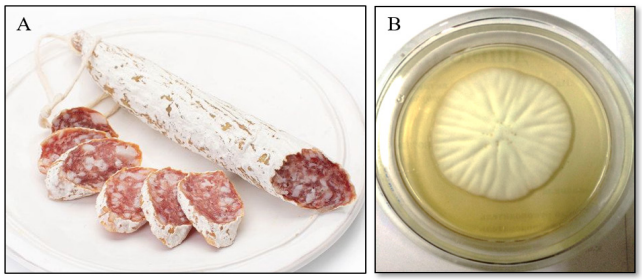

Figure 1 – Isolation of a pure *P. candidum* strain: A – Sausage product (salami), B – *P. candidum*.

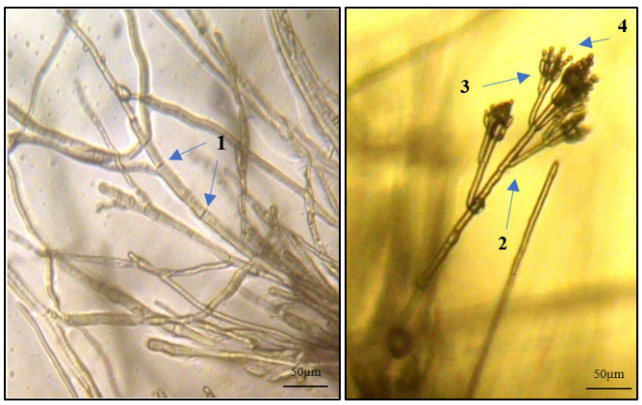

Figure 2 – Microscopic examination of the *P. candidum* strain at 40× magnification: 1 - Septate mycelium, 2 - Conidiophores, 3 - Biverticillate phialides (metulae), 4 - Chains of conidia.

has a white septate mycelium and conidiophores shaped like brushes. These growth characteristics and the structure of the mycelium are specific to the *Penicillium* species.

Study of the cultural properties of the P. candidum strain.

When the fungus was grown in liquid nutrient media, several characteristic features were observed. These include the formation of dense, medium-sized globules within the medium, the absence of surface growth, and the lack of wall-adhering growth during the first 3 days. The nutrient medium remained clear throughout the cultivation period (Figure 3).

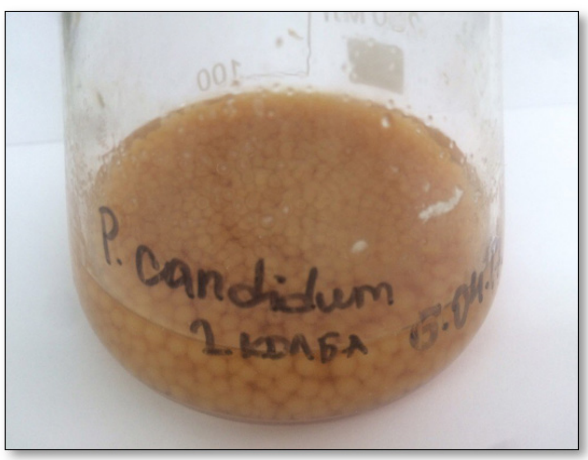

Figure 3 – Cultivation of the dermatophyte *P. candidum* in liquid nutrient media (mycelial growth characteristics at 2–3 days).

As shown in Figure 3, medium-sized, dense mycelial glob-

ules of *P. candidum* are present within the nutrient medium. This growth pattern is associated with the specific characteristics of mycelial development, and under continuous agitation during cultivation in liquid nutrient media, mycelial globules are formed.

To study the cultural properties of the *P. candidum* strain, inoculations were also performed on solid nutrient media: Sabouraud agar, Czapek medium, potato agar, corn agar, and Sabouraud broth. Colony growth monitoring using various nutrient media was carried out over a 15-day cultivation period. The growth of the strain on solid nutrient media was carefully examined and described on the 5th day of cultivation, at which point all mycelial growth characteristics and biochemical properties related to the breakdown of nutrient medium components became evident.

On Sabouraud agar, *P. candidum* forms colonies of a creamy color by the 5th day, with a velvety, smooth surface, a raised, densely powdery center, and a yellow color on the reverse side of the colony. Noticeable radial grooves were observed (Figure 4).

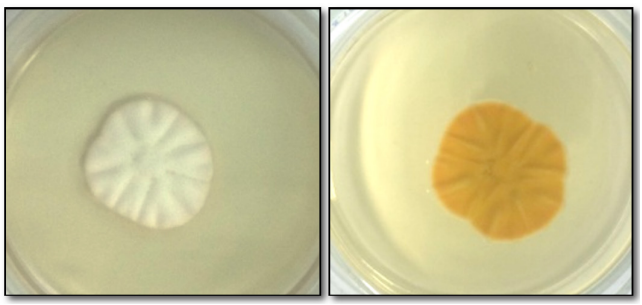

Figure 4 – Growth characteristics of *P. candidum* on Sabouraud agar.

When grown on Czapek medium, *P. candidum* forms a white, oval-shaped colony. The surface is smooth with a raised center (Figure 5). The reverse side of the colony is yellow in color.

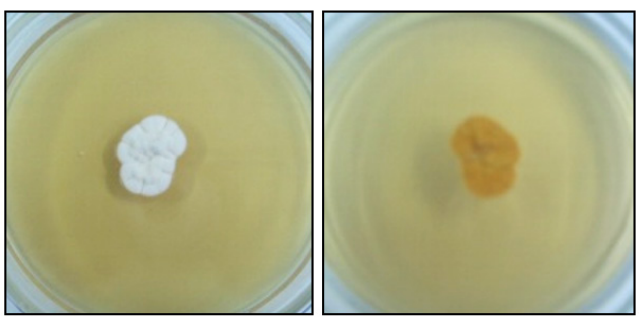

Figure 5 – Growth characteristics of *P. candidum* on Czapek medium.

The use of potato agar for cultivating the *P. candidum* strain resulted in the formation of a white colony with radial grooves extending from the center of the colony. The surface is smooth with even edges (Figure 6). The reverse side of the colonies has a honey color and a wrinkled surface.

The preparation of nutrient medium based on corn agar was characterized by the growth of the *P. candidum* strain as a white colony slightly protruding above the surface of the nutrient medium. An embedded growth of the mycelium within the medium was observed, causing the color of the medium to change from milky to creamy as the mycelium developed.

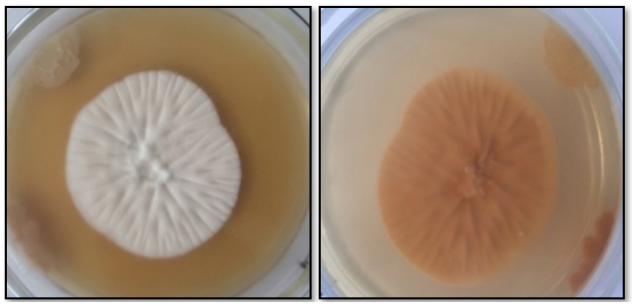

Figure 6 – Growth characteristics of *P. candidum* on potato agar.

The colony showed a slight elevation at the center, with uneven, ragged edges (Figure 7). The reverse side of the colony exhibited a creamy-pink color.

When grown on honey agar, *P. candidum* exhibited a characteristic mycelial colony growth: a white colony with a slightly pinkish hue and a small yellow pigment at the center of the colony. Radial grooves were prominently expressed during colony formation (Figure 8).

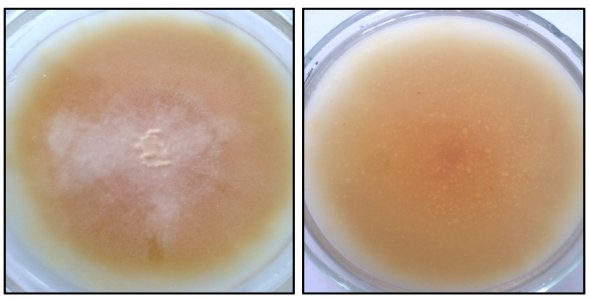

Figure 7 – Growth characteristics of *P. candidum* on corn agar.

The reverse side of the colony had a red-orange color with a wrinkled surface.

Thus, the use of various nutrient media provided results showing that the best mycelial colony growth of *P. candidum* was observed on potato and honey agar. This may be due to the presence of easily digestible monosaccharides in the composition of these media, which are essential for the development of the fungal mycelium. The use of Sabouraud, Czapek, and corn agar did not result in rapid mycelial growth and was characterized by changes in the growth pattern and colony color.

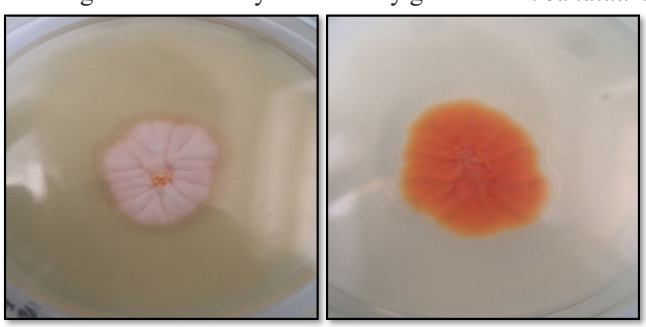

Figure 8 – Growth characteristics of *P. candidum* on honey agar.

Biochemical properties of the P. candidum strain.

The saccharolytic activity of the *P. candidum* strain was studied using standard Giss media containing sucrose, glucose, lactose, maltose, and mannitol. During the experiment,

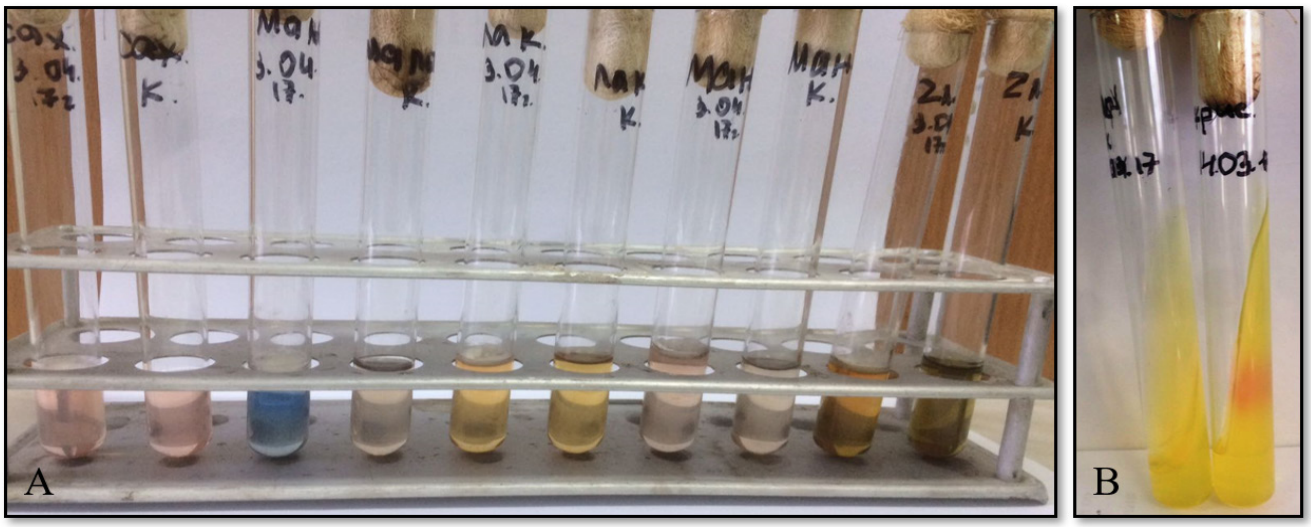

Figure 9 – Saccharolytic properties of the *P. candidum* strain: A – Giss media, B – Christensen medium.

it was found that the saccharolytic properties of the fungus are quite diverse. The studied *P. candidum* strain exhibits moderate enzymatic activity with respect to sugars. It breaks down glucose, maltose, and weakly hydrolyzes lactose. Visually, this was indicated by a change in the color of the nutrient medium with varying intensity and the formation of gas bubbles (Figure 9).

As shown in Figure 9A, in the test tube with sucrose, the medium has a pale pink color, with white colonies of *P. candidum* growing on the surface, while the control remains pink. The maltose medium is blue, with white colonies on the surface, and no changes in the control. The lactose medium is yellow, with white colonies and an unchanged control. The mannitol medium is pale pink. The glucose medium is dark yellow, with visible growth of the strain on the surface, while the control is brownish-green. Thus, *P. candidum* demonstrates significant activity in the breakdown of glucose and maltose and exhibits proteolytic activity.

As shown in Figure 9B, the analysis of *P. candidum* growth on Christensen medium reveals active urea hydrolysis with the release of ammonia, which alkalinizes the medium, resulting in a color change from yellow to pink-red. The color change of the nutrient medium indicates positive urease activity of the studied *P. candidum* strain. Since the medium contains 5% urea, it can be concluded that the *P. candidum* strain is capable of breaking down urea. Thus, the *P. candidum* strain exhibits pronounced saccharolytic properties and urease activity.

Study of the antagonistic properties of the P. candidum strain.

According to literature data, industrial fungal strains are capable of producing active substances, including various types of antibiotics. This property allows certain strains to exhibit antagonistic activity against foreign pathogenic microflora and preserve the original characteristics of the product.

In this context, we conducted a study of the antagonistic properties of the *P. candidum* strain. The concentration of active metabolites (antibiotics) produced by the strain was determined using the diffusion method in agarized media (Figure 10).

As shown in Figure 10, the *P. candidum* strain exhibits antagonistic properties against *Bacillus* spp., with a growth inhibition zone diameter of 24 mm. Given that this strain secretes antibiotic-class substances, using a specialized recalculation method based on the inhibition zone diameter, the antibiotic concentration was calculated to be 10.6 µg/mL. Thus, it can be concluded that the studied *P. candidum* strain has antagonistic activity against *Bacillus* spp., with the concentration of the active substance being 10.6 µg/mL.

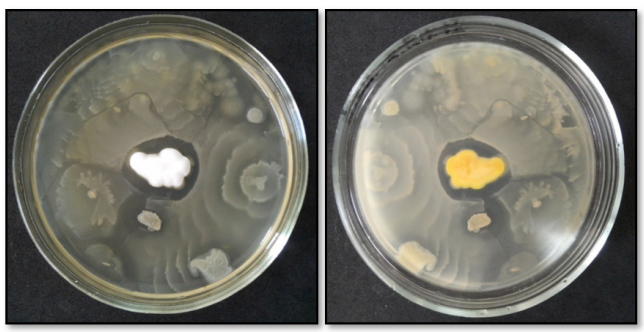

Figure 10 – Determination of the antagonistic properties of the *P. candidum* strain.

4 DISCUSSION

Morphological, cultural, and biochemical properties. The results of studying the morphological, cultural, and biochemical properties of the *P. candidum* strain confirm its potential as a promising microbiological tool in the food industry. The observations made during the experiments are consistent with international data, which emphasize the importance of optimizing cultivation conditions to enhance the production characteristics of this microorganism.

Morphological and cultural characteristics. The findings indicating significant differences in colony morphology on various nutrient media align with the conclusions of Visagie, C.M. et al., who noted that medium parameters substantially influence the structure of the mycelium and morphology of *P. camemberti*, a closely related species of *P. candidum* [23]. The differences in radial grooves and colony texture observed in this study confirm the fungus's flexibility and adaptability to different substrates.

Biochemical properties. The study of saccharolytic activ-

ity showed that *P. candidum* breaks down glucose and maltose, which is consistent with the findings of Gibbons et al., who highlighted the importance of carbohydrate metabolism in the maturation of cheese using *Penicillium* [4]. Additionally, the urease activity of this strain confirms its role in biochemical transformations related to protein and lipid degradation, as previously described in the works of Ropars et al. [5].

Antagonistic properties. The antagonistic activity of the *P. candidum* strain against *Bacillus* spp. is a significant result that underscores the potential of using this fungus as a bio-preservative agent. Similar properties were observed in the studies of Lessard, M.H., where it was noted that the metabolic products of *Penicillium* can suppress the growth of pathogenic microflora, ensuring the safety of the final product [24]. The antibiotic substance concentration of 10.6 µg/mL confirms the effectiveness of this approach.

The results obtained confirm the high adaptability and biochemical activity of *P. candidum*. These findings are consistent with international research, strengthening the scientific basis for its further application in the food industry.

CONCLUSION

For the accumulation and rapid growth of *P. candidum* biomass, potato and honey agar are the most suitable media. As an additional carbohydrate source, glucose or maltose can be added. The cultural properties of the strain and the nature of colony growth depend on the composition of the nutrient medium. Microscopic examination of the *P. candidum* strain revealed the presence of septate mycelium and conidiophores characteristic of this species. The saccharolytic ability of the *P. candidum* strain was studied using standard Giss media: the strain shows moderate enzymatic activity with respect to sugars. It breaks down glucose, maltose, and weakly breaks down mannitol. The presence of positive urease activity and the ability to break down urea have been proven. The *P. candidum* strain exhibits antagonistic properties against *Bacillus* spp., with the concentration of the active substance being 10.6 µg/mL.

CONFLICT OF INTEREST

There are no conflicts of interest to declare.

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PENICILLIUM CANDIDUM ШТАМЫНЫҢ КУЛЬТУРАЛЬДЫҚ ЖӘНЕ БИОХИМИЯЛЫҚ ҚАСИЕТТЕРІН ЗЕРТТЕУ

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АҢДАТПА

Бұл жұмыста *Penicillium candidum* штамының морфологиялық, культуральдық және биохимиялық қасиеттерін зерттеу нәтижелері ұсынылған. Штамм колбасалық өнімнен (салями) бөлініп алынды. Өсу сипаты мен мицелий құрылымын бағалау үшін түрлі қоректік орталарда өсірілді. Қатты орталарда колониялардың түсі, пішіні және беткі құрылымында айырмашылықтар байқалды. Ең жақсы өсу картопты және балды агарларда анықталды, мұнда колонияларда радиалды ойықтар мен қатпарлы беткі қабат пайда болды. Сұйық ортада беткі және қабырғалық өсу байқалмай, тығыз мицелий глобулалары түзілді. Биохимиялық зерттеулер *Penicillium candidum* штамының глюкоза мен мальтозаны ыдыратып, лактозаны әлсіз ферменттейтінін және уреаза белсенділігін көрсететінін анықтады. Антагонистік сынақтарда штамм *Bacillus* spp. өсуін тежеп, 24 мм диаметрде өсу аймағының болмауын көрсетті. Антибиотикалық зат концентрациясы 10,6 мкг/мл құрады. Осылайша, *Penicillium candidum* штамы жоғары биохимиялық белсенділік пен антагонистік қасиеттер көрсетіп, оны тағам өнеркәсібінде биоконсервант немесе технологиялық мәдениет ретінде қолдануға әлеуеті бар екенін дәлелдейді.

Негізгі сөздер: *қоректік орталар, Penicillium candidum, морфологиялық қасиеттер, культуральдық қасиеттер, антагонистік қасиеттер.*

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ИЗУЧЕНИЕ КУЛЬТУРАЛЬНЫХ И БИОХИМИЧЕСКИХ СВОЙСТВ ШТАММА *PENICILLIUM CANDIDUM*

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АННОТАЦИЯ

В работе представлены результаты изучения морфологических, культуральных и биохимических свойств штамма *Penicillium candidum*, выделенного из колбасного изделия (салями). Штамм культивировали на различных питательных средах для оценки характера роста и структуры мицелия. На твердых средах наблюдались различия в цвете, форме и поверхности колоний. Наилучший рост отмечен на картофельном и медовом агарах, где колонии имели радиальные борозды и складчатую поверхность. В жидких средах образовывались плотные глобулы мицелия при отсутствии поверхностного и пристеночного роста. Биохимические исследования показали, что *Penicillium candidum* разлагает глюкозу и мальтозу, слабо ферментирует лактозу и обладает уреазной активностью. В антагонистических тестах штамм проявил способность ингибировать рост *Bacillus* spp., формируя зону отсутствия роста диаметром 24 мм. Концентрация антибиотического вещества составила 10,6 мкг/мл. Таким образом, штамм *Penicillium candidum* демонстрирует высокую биохимическую активность и антагонистические свойства, что подтверждает его потенциал для применения в пищевой промышленности как биоконсервант или технологическая культура.

Ключевые слова: *питательные среды, Penicillium candidum, морфологические свойства, культуральные свойства, антагонистические свойства.*