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FEATURES OF GROWTH OF FUNGI OF THE GENUS CANDIDA ON THE NUTRITIONAL BASIS OF RICE BRAN

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ANNOTATION----------

Obtaining a cheap nutrient medium and a base that would provide the medium with good nutritional qualities is of great practical importance (Iskhakova Kh.I., 1998; Baronets N.G., 2003; Nuraliev N.A. et al., 2004). It is impossible to prepare a nutrient medium with the same nutritional value for all pathogenic microbes due to the extremely diverse and non-individual needs of individual types of microorganisms. In addition to fulfilling the basic requirements for nutrient media, they must be cost-effective and for nutrient media, they must be cost-effective and easy to handle in bacteriological studies (Nuraliev N.A. and co-authors, 2003; Iskhakova Kh.I. and co-authors , 2004).

Given the fact that yeast-like fungi of the genus Candida develop well and quickly on dense agar media with carbohydrates (Sokolova V.G., 1970; Bazhenov L.G., Artemova E.V., 2004), the aim of the work was to develop a new nutritional basis for and cultivation based on rice bran.

KEY WORD: ***genius, candida, rice, bran, water, bottom, pathogenic microbes.----------***

MATERIALS AND METHODS

We have conducted exploratory research, as a result of which we have developed a technology for preparing a nutritional base based on rice bran. The raw material is rice bran, obtained in the process of rice processing. They are a very cheap waste from rice cultivation and very rich in nutritional and biological value.

GET METHOD

Rice bran was soaked in distilled water at a temperature of 10-12ºC. Before soaking, the water was averaged with 1 N NaOH solution to pH 7.8–8.0. The temperature (10-12ºC) was maintained throughout the extraction, 80 g (8.0%) of rice bran and 2.5 g (0.25%) of NaCl were added per 1 liter of the hearth. Soaking of rice bran and further cooking was carried out in acid and alkali resistant dishes. It is convenient to use glass bottles with a volume of 20 liters for preparing a large amount of medium. Nutrients from rice bran were extracted for 24 hours.

In the first 8-9 hours, the rice-and-brand mixture settling to the bottom of the tank was mixed 4-5 times with water. In the next 15-16 hours, the bottle, together with the contents, was left completely alone for better sedimentation of suspended particles to the bottom. After 15-16 hours, carefully, so as not to capture the settled rice bran from the bottom, the settled water was drained along with the rice bran proteins in the hydrosol state and other extracted nutrients. Subsequently, the mood of rice bran was hydrolyzed with pepsin and pancreatin. At the end of treatment with pancreatin, the hydrolyzate was stirred, a 1 N HCl solution was added and the pH was adjusted to 5.0-5.2, boiled for 15 minutes and allowed to settle for 20 minutes. The precipitate formed was filtered through a cotton-gauze filter, alkalized with 1 N NaOH solution to pH – 8, 0 was boiled again and filtered again through a cotton-gauze filter until clear. Then the required pH was adjusted, boiled again and filtered through a cotton-gauze filter. Poured into working utensils and sterilized in an autoclave at 0.8-1.0 atm for 30 minutes. The losses of the medium due to boiling were compensated.

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The studies were carried out at the Department of Microbiology and Epidemiology of the Urgench branch of the Tashkent Medical Academy and the Research Institute of EMIZ of the Ministry of Health of the Republic of Uzbekistan using generally accepted bacteriological methods, with simultaneous bacteriological control of the quality of the nutrient medium. The identification of microorganisms was carried out before Bergi (1997). The obtained material was statistically processed on a personal computer.

RESEARCH RESULTS AND DISCUSSION

We have carried out a series of comparative experiments to test the nutritional base. The possibility of cultivating yeast-like fungi of the genus Candida on a nutrient basis containing rice bran as a basis was studied.

The basis of the nutrient medium was obtained by water extraction followed by drying (evaporation). NaCl was added to the resulting dry residue up to 0.5% concentration and furacillin to inhibit the growth of gram-positive and gram-negative flora, pH was set in the range of 5.0-5.2. A mixture of pathogenic and opportunistic microorganisms with test strains of yeast-like fungi of the genus Candida was inoculated onto the prepared dense nutrient medium. In total, 7 different two-component mixtures were studied: E.coli, P.aeruginosa, P. Vulgaris, S.typhimurium, S.aureus, S.epidermidis, E.faecalis.

It has been established that the medium based on rice bran with pH 5.0-5.2 has elective properties, gives predominant growth of fungi of the genus Candida when cultivated under aerobic conditions, at 37º C for 24 hours, followed by 24 hours of incubation at room temperature. P.aeruginosa is also cultivated under these conditions.

One of the stages of testing the base was to determine the yield of wet and dry myrrh mass on our proposed and commercial media. Of the tested strains, No. 1-4 were reference and No. 5-8 were hospital strains of Candida sp isolated from patients in real time. The amount of inoculum was the same for all studied strains - 5*10 5 mg/ml. The yield of wet and dry microbial mass was determined in a washout of a 48-hour culture of reference and hospital strains of Candida sp grown in Petri dishes on plate agar. The microbial mass was collected by centrifugation, washed twice with saline followed by, after which the wet mass was weighed. To determine the dry bacterial substance, the wet mass was treated with acetone, collected in a centrifuge tube by centrifugation,

The difference between the yield of the bacterial mass from the test and control media was: for the wet mass of strain No. 1 109.9 mg ($P<0.001$), for the dry mass 25.3 mg ($P<0.001$); strain #2 - 81 mg ($P<0.05$); 22.5 mg ($P<0.001$); strain No. 3 - 44.3 mg ($P<0.05$), 18.2 mg ($P<0.05$); strain No. 4 - 47.2 mg ($P<0.05$), 6.3 mg ($P<0.05$); strain No. 5 - 30.6 mg ($P<0.05$), 9 mg ($P<0.05$); strain No. 6 - 80.1 mg ($P<0.05$), 16.3 mg ($P<0.05$); strain No. 7 - 95.3 ($P<0.001$), 2.2 mg ($P<0.05$); strain No. 8 - 71.6 ($P<0.05$), 2.3 mg ($P<0.05$).

Of the 8 strains studied on the medium of rice bran, significantly more wet bacterial mass came out in 75.0% of cases; for dry in 50.0% of cases. Reference strains and hospital cultures did not differ from each other in terms of wet bacterial mass yield (75.0% of cases). By dry weight, museum strains for a higher yield of 75.0%, and hospital strains of 25.0%. This seems to be due to the fact that the reference strains are adapted to artificial environments, as a result of their storage of frequent subcultures.

One of the conducted studies was also an experimental study of the selective properties of rice-and-tube water extract (ROVE). It was prepared in two versions; the first on isotonic 0.5% NaCl solution and the second on nutrient broth.

Prepared two and three component mixed cultures of reference strains of Candida sp with gram-positive and gram-negative microorganisms. The microbial suspension was prepared in an isotonic NaCl solution at concentrations of 10^4mg/ml and 102 mg/ml. A total of 6 mixed cultures were prepared: Candida sp c E. coli; Candida sp c S.aureus; Candida sp c P.aeruginosa; Candida sp cc S.epidermidis; Candida sp c E.coli and S.aureus; Candida sp c E. coli and S. epidermidis. Inoculations of mixed cultures on prepared nutrient media were incubated in a thermostat at 37°C. The results were recorded after 24 and 48 hours according to the nature of growth (cultural properties) and smear microscopes when stained by Gram.

It was found that after 24 hours there was no visible growth on plate agar in all the inoculations, but after 48 hours the growth of colonies was detected. After their counting and identification, it was shown that there was a visible growth of Candida sp on ROVE, but mixed cultures of gram-positive and gram-negative cultures did not give growth, except for P. auruginosa in both variants of the medium. The growth of Candida sp was noted in both variants of ROVE, but no significant differences in quantitative and qualitative growth were found in them. When sowing museum cultures of fungi of the genus Candida, it was found that the nature of their growth on an experimental basis is identical to the growth on commercial Sabouraud medium. Studies have established 3 types: colonies are round, creamy, whitish or grayish, convex, shiny; colonies are round with jagged edges, whitish-gray with a raised knobby center, with smooth radially folded uneven edges; colonies white or gray, dryish, viscous consistency, uneven edges, finely folded. On microscopy, the cells are round or

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ovoid, of various sizes - ground cells are small, mature cells are larger, in the form of characteristic budding forms.

CONCLUSIONS

1. An elective nutritional base based on rice bran is proposed, which is suitable for the cultivation of fungi of the genus Candida and gives typical cultivation and morphological characteristics of the microorganism.
2. From the studied strains on the medium of rice bran, significantly more wet bacterial mass came out in 75.0% of cases; for dry in 50.0% of cases. Reference strains and hospital cultures did not differ from each other in terms of wet bacterial mass yield. By dry weight, museum strains gave a higher yield of 75.0% and hospital strains of 25.0%.
3. Visible growth of Candida sp takes place on the rice-tuber water extract, but mixed cultures of gram-positive and gram-negative cultures seeded together did not give growth, except for P. aeruginosa in both variants of the base. The growth of Candida sp was noted in both variants, but no significant differences in growth were found on them.
4. The use of the proposed nutritional base based on rice bran in the practice of bacteriological and mycological laboratories will create great cost savings.

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