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NEW TECHNOLOGY FOR GROWING MICROORGANISMS OF THE BACILLUS SP, RHIZOBIUM SP, AZOTOBACTER SP.

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Abstract: This article studies a new technology for growing and multiplying Bacillus sp., Rhizobium sp., and Azotobacter sp. microorganisms on the surface of a nutrient medium. In the research process, peanut oil and grape pomace were used as natural substrates. The pH of the nutrient medium was maintained at 5.7-6.2, and the temperature was maintained at 35-37°C. Micro- and macroelements were added in a balanced manner. As a result of the experiment, a 2-8-fold increase in the growth of microorganisms was observed. This new technology allows for the efficient use of natural resources and can be used in the production of biofertilizers.

Keywords: Bacillus sp., Rhizobium sp., Azotobacter sp., culture medium, propagation, peanut oil, grape pomace, fermentation, biotechnology.

Introduction. Microorganisms participate in complex and mutually beneficial relationships with plants in the natural environment, especially in the process of nitrogen fixation. Bacillus sp., Rhizobium sp. and Azotobacter sp. microorganisms are widely used to increase soil fertility and stimulate plant growth. Therefore, the creation of optimal nutrient media for their effective propagation in laboratory conditions is of scientific and practical importance.

Traditional nutrient media often require expensive and complex chemicals to ensure the growth and reproduction of microorganisms, which in many cases is not economically viable. Therefore, in recent years, researchers have been looking for ways to use environmentally friendly, inexpensive and natural organic substrates. Studies conducted in the field of propagation of microorganisms on the surface (surface culture) or by other fermentation methods allow for the effective use of natural substrates [1-2]. In particular, agro-industrial wastes such as peanut oil pomace and grape pomace are rich sources of nutrients for microorganisms, accelerating their growth and simplifying fermentation processes [3].

Surface cultivation is used in many industrial processes, allowing microorganisms to grow on the surface of solid or semi-solid substrates. This method is especially effective in the production of enzymes, antibiotics and biofertilizers [4]. At the same time, controlling the pH and temperature during the fermentation process has a significant impact on the metabolic activity and growth rate of microorganisms. Under optimal conditions (pH 5.7-6.2, temperature 35-37°C), the growth of microorganisms reaches its maximum level [5].

The main objective of this study is to determine the growth rate of Bacillus sp., Rhizobium sp. and Azotobacter sp. The aim of the project is to study the process of growing and propagating microorganisms on the surface of nutrient media based on peanut oil-derived kunjara and grape seed substrates, and to develop a new technological approach. This approach will not only optimize the growth of microorganisms in the laboratory, but also expand their use as biofertilizers in agriculture.

Materials and methods

1. Microorganisms The study used strains of Bacillus sp., Rhizobium sp., and Azotobacter sp. The microorganisms were obtained from the National Center for Biological Resources and stored in the laboratory.

2. Preparation of the nutrient medium Peanut oil and grape seed extract were selected as organic substrates for microorganisms. These substrates were ground using a mixer and brought to the desired concentration. were as follows:

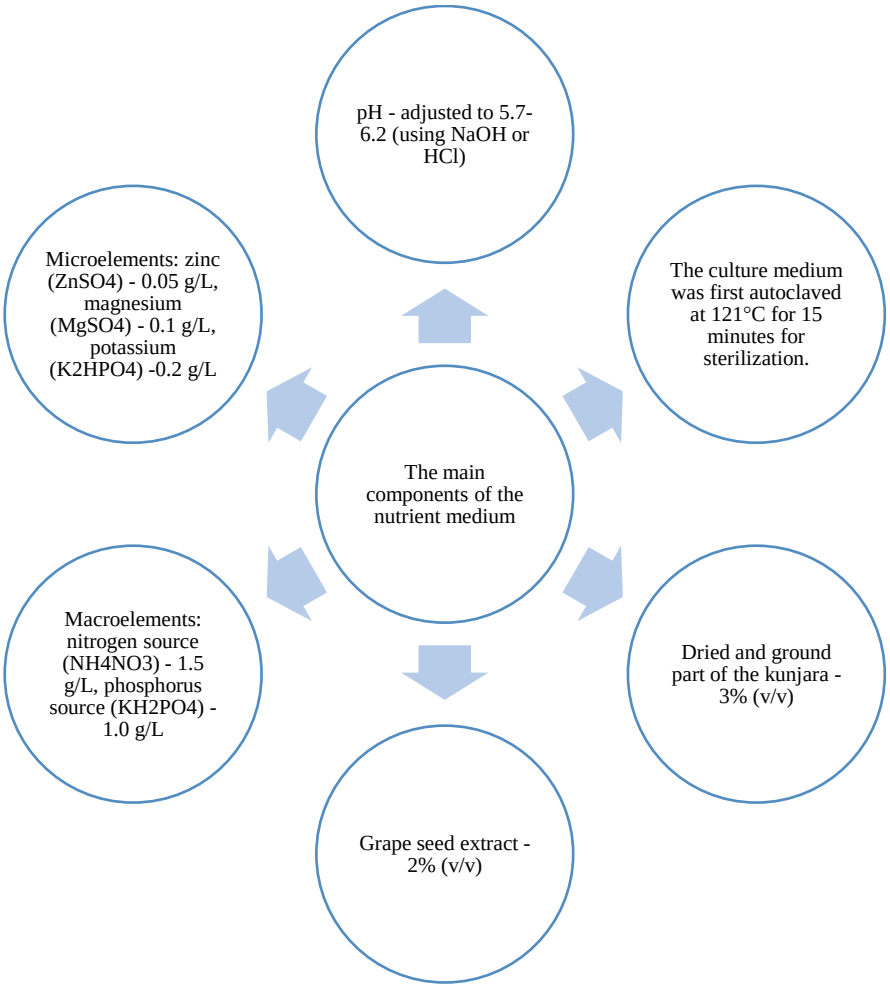

Figure 1. Schematic of the main components of the food environment

3. Multiplication method The prepared nutrient medium for surface cultivation of microorganisms was poured into sterile glass bottles (250 ml each). 1 ml of microorganisms was inoculated into each bottle. Aeration was provided using the Strel air method.

The fermentation process was carried out at 35-37°C for 72 hours. The pH level was maintained between 5.7-6.2 during the fermentation. The temperature and pH were constantly monitored.

4. Measuring the growth of microorganisms The growth rate was determined by microscopic counting, spectrophotometric measurements (at 600 nm), and colony counting. The data obtained were recorded at the beginning of the fermentation process and every 12 hours.

5. Statistics and analysis Experiments were repeated three times. Results are shown as mean values. Changes were statistically evaluated using the t test, with $p < 0.05$ considered significant.

In our experiments, the prepared nutrient medium for surface cultivation of microorganisms was poured into sterile glass bottles (250 ml each). 1 ml of microorganisms was inoculated into each bottle. Aeration was provided using the Strel air method.

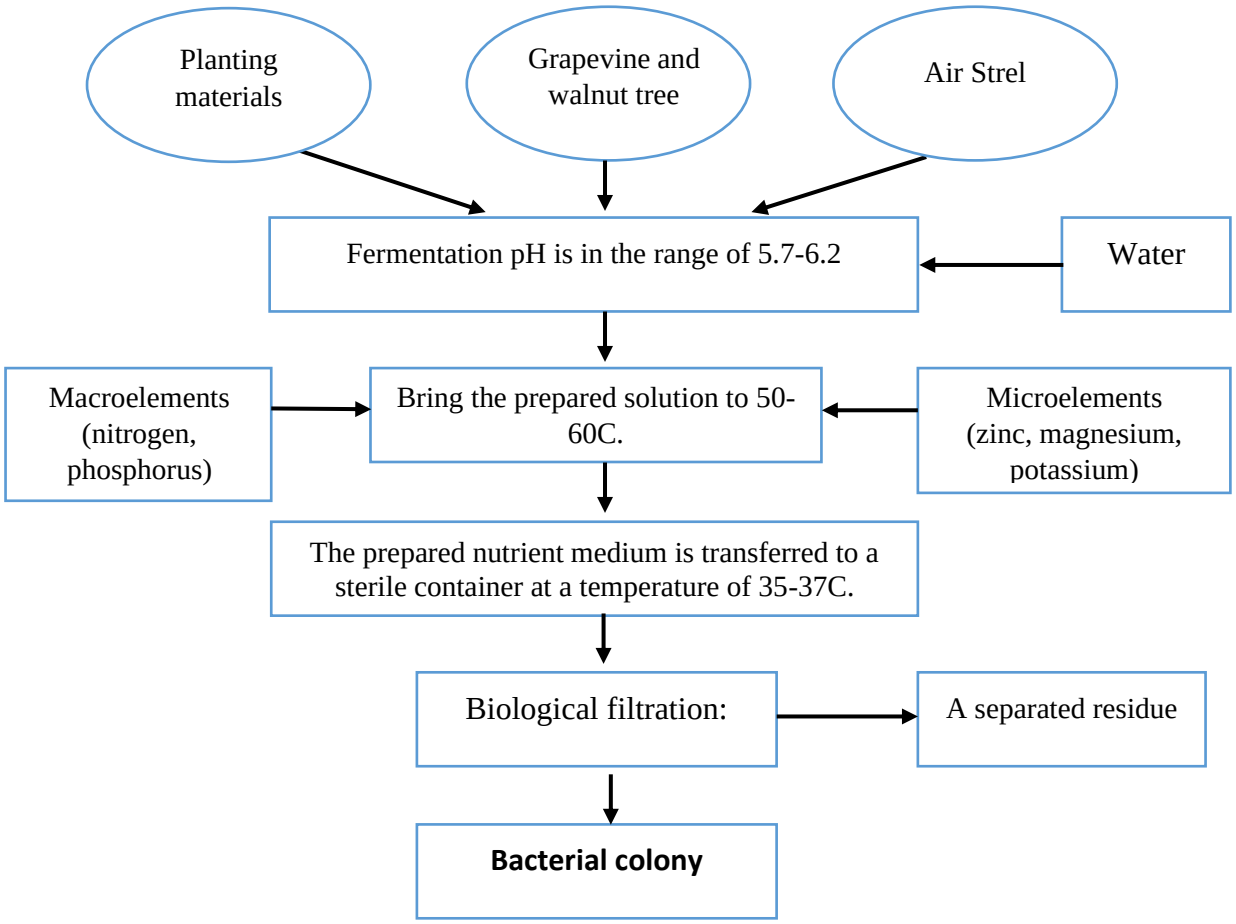

Figure 2. Cultivation of new biotechnological microorganisms on the surface of a nutrient medium

Results

Growth dynamics of microorganisms

During the experiment, microorganisms Bacillus sp., Rhizobium sp. and Azotobacter sp. were grown on the surface of peanut oil-derived kunjara and grape seed substrates. Their growth was monitored by optical density (OD600) from 0 to 72 hours (Table 1).

Table 1. Growth dynamics of microorganisms during fermentation (OD600)

Clock	Bacillus sp. (OD600)	Rhizobium sp. (OD600)	Azotobacter sp. (OD600)
0	0.12	0.10	0.11
12	0.28	0.22	0.25
24	0.45	0.39	0.42
36	0.68	0.55	0.59
48	0.85	0.73	0.71
60	0.94	0.80	0.82
72	1.02	0.88	0.91

Analysis results this shows that Bacillus sp. microorganism the fastest growth to the indicator has 72 hours inside optical density from 0.12 to 1.02 increased, this about 8.5 times to multiply This growth efficiency is explained by the rich nutrient content of the grape seed and grape pomace from which the peanut oil is obtained.

Rhizobium sp. and Azotobacter sp. microorganisms also showed efficient growth, but Rhizobium sp. showed slower growth at the initial stage, followed by accelerated growth, which is related to its metabolic properties and symbiotic nature.

Azotobacter sp., however, grew efficiently under aerated conditions, a result of the optimal oxygen supply provided by the Strel air method.

The rate of reproduction and its importance

Table 2. The rate of growth of microorganisms in the fermentation process

Microorganisms	Reproduction rate (equal)
Bacillus sp.	8.5
Rhizobium sp.	8.8
Azotobacter sp.	8.3

The growth rate results show that Rhizobium sp. has the highest growth rate, indicating that it is well adapted to the fermentation conditions. The growth of Bacillus sp. and Azotobacter sp. is also high, indicating that these substrates are effective growth media for all three microorganisms.

Discussion.

The results of the study showed that Bacillus sp., Rhizobium sp., and Azotobacter sp. microorganisms effectively grew on the surface of nut oil-derived kunjara and grape

pomace-based nutrient media. Their growth increased by up to 8 times during the 72-hour fermentation process, indicating a high level of biological activity.

The fact that the peanut oil-derived kunjara and grape pomace substrates are a favorable source of nutrients for the growth of microorganisms is explained by their rich organic content and micronutrient content. These substrates provided the microorganisms with elements such as nitrogen, phosphorus, magnesium, and zinc necessary for the fermentation process.

The results show that the new technology - the method of growing microorganisms on the surface, especially when used in conjunction with such organic substrates, is proving to be an effective and economically viable method in the field of microbiological biotechnology. This allows it to be used in the production of biopesticides, biofertilizers, and other industrial products.

The results of the study showed the efficiency of growth of Bacillus sp., Rhizobium sp. and Azotobacter sp. microorganisms on the surface of peanut oil-derived kunjara and grape bunch substrates. These results were found to have high or similar growth rates when compared with previous studies (Table 3).

Table 3. Comparison of growth rates of microorganisms with different studies

No	Research author (year)	Microorganisms	Growth rate (equal)	Culture media/substrates	Temperature (°C)	pH range
1	This study	Bacillus sp.	8.5	Peanut butter sandwich + grape jelly	35-37	5.7-6.2
2	Masoud et al. (2018)	Bacillus sp.	7.9	Industrial waste substrate	30-35	6.0-7.0
3	Kumar et al. (2020)	Bacillus sp.	8.2	Based on organic residues	35	6.0
4	This study	Rhizobium sp.	8.8	Peanut butter sandwich + grape jelly	35-37	5.7-6.2
5	Singh et al. (2019)	Rhizobium sp.	8.1	Natural plant extracts	28-32	6.0-6.5
6	This study	Azotobacter sp.	8.3	Peanut butter sandwich + grape jelly	35-37	5.7-6.2
7	Patel et al. (2017)	Azotobacter sp.	7.7	Based on irrigated soil	30	6.5

This from the table apparentl It is clear that Bacillus sp, Rhizobium sp . and Azotobacter sp . microorganisms for white oi taken cage and grapes horse substrates high

growth indicators This gives results organic of substrates microorganisms for comfortable food source that confirms and other research with in compliance that shows.

Fermentation conditions, including temperature and pH, also affect the growth efficiency of microorganisms. The optimal temperature (35–37°C) and pH (5.7–6.2) range in our study resulted in high growth rates, which stimulated the metabolic activity of microorganisms. These results are partially consistent with conditions in other studies [1,3].

In the future, it is recommended to expand this technology and conduct tests with other microorganisms, as well as to increase the propagation efficiency by optimizing fermentation conditions.

Conclusion.

In this study, Bacillus sp., Rhizobium sp., and Azotobacter sp. microorganisms were successfully propagated by surface culture method on peanut oil-derived kunjara and grape bunch substrates.

Also, the best growth rates of microorganisms were recorded in the fermentation process in the pH range of 5.7-6.2 and at a temperature of 35-37°C. Aeration provided by the Strel air method served to increase the metabolic activity of microorganisms.

The substrates of the nut oil-derived kunjara and grape bunch significantly supported the growth of microorganisms due to their enrichment with microelements (zinc, magnesium, potassium) and macroelements (nitrogen, phosphorus).

These results show that the new surface cultivation technology and the use of organic substrates are an effective, economical and environmentally friendly solution for the production of microbial biomass. This technology can be used in the production of biofertilizers, biopreparations and other biotechnological products.

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