Search for antagonists to protect plant raw materials from pathogens

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Abstract. The paper presents the results of research on the selection of biological agents with antagonism to microorganisms affecting raw materials of plant origin. The antagonists selected at the first stage showed significant changes in the antagonistic activity of various isolates when the temperature of the medium increased to 45°C. There was no decrease in activity in isolate 20, so it is resistant to high temperature. Isolate №21 had a significant increase in antagonistic activity, it is likely to be more resistant to high temperatures and some biochemical enzymatic processes are stimulated, leading to increased antagonistic properties. And in isolates 9, 16 and 23, on the contrary, it was lowered, possibly due to the fact that the increased temperature causes structural and biochemical changes in the cell of the microorganism. Similarly, isolated isolates №9, 16, 23, 21, and 15 had high antagonistic activity against micromycetes that contaminate plant-based feed.

1. Introduction

Currently, a significant part of the silage being harvested remains of poor quality. In 1 kg of dry matter on average contains 0.70 feed units, with a content of 8-11% of raw protein. At the same time, in the conditions of a sharp reduction in the number of livestock, with the need for animal husbandry in digestible protein of about 46 million tons, 10.5 million tons are actually consumed. The deficit of digestible protein in coarse and juicy feeds is 0.7; in concentrated - 0.4 million tons. Therefore, the issue of harvesting high-quality silage is very relevant, and its preparation with the use of various preservatives is one of the promising techniques in the chain of the technological process of forage preparation, which should ensure the development of lactic acid microflora in the plant mass and the suppression of the growth of putrid, oily bacteria[1, 2, 3, 4], molds and fungi. In order to preserve plant-based feed, various methods are used to protect feed raw materials, including chemical methods that allow you to prepare silage from any crop[5, 6, 7, 8, 9, 10]. The search for new biofungicides, cheaper, adapted for automated application [11, 12, 13, 14], harmless and having sufficient preserving
power, is an urgent task \[15, 16, 17, 18\]. Therefore, along with chemical preservation, it is of particular interest to use for these purposes microorganisms that have the ability to produce bactericidal and antifungal (anti-mold) substances\[19, 20, 21, 22, 23\].

One of the high-quality methods of foraging is the production of high-quality silage, which occupies a significant share in the ruminant rations in the winter-stall period. Currently, a new, very promising direction in the conservation of green feed is being successfully developed in the system of developing rational silage techniques—the use of environmentally friendly and harmless biological preparations for the animal body (bacterial starter cultures and enzyme preparations) \[24, 25, 26, 27, 28\].

In this regard, the purpose of our research was to select microorganisms that are active antagonists to pathogenic bacteria.

2. Materials and approach

Isolates were isolated from a wide range of samples of agricultural and food products, as well as from biological preparations for agricultural purposes to test their biological and antagonistic activity in order to expand and strengthen the conservation capabilities and improve the quality of silage starter culture.

Pure cultures were isolated by homogenization of samples in sterile saline solution and cultivation in Petri dishes at 30-45°C on meat-peptone agar (MPA), hydrolyzate-milk medium (HMS), MRS medium under thermostat conditions for 48 hours and transplanting from individual grown colonies to new cups. The antagonistic activity of isolates against certain bacteria that contaminate plant-based feed was determined using agar block methods. Using the method of agar blocks, the isolate was sown on the surface of meat-peptone agar (Mrs, HMS medium) in a Petri dish and thermostated at 37°C until a "solid lawn" was formed \[29, 30\]. Then, using a sterile cork drill, blocks were cut out of it, which were transferred to the pre-seeded pathogen test culture surface of the MPA (Chapek medium) in another Petri dish. The test culture was sown with a spatula, agar blocks were placed growing up at an equal distance from each other and from the edges of the Cup, pressing tightly to the surface of the medium. The control was the cultivation of the pathogen without an isolate. The cups were incubated in a thermostat at 37°C. Experience was recorded on days 2, 4 and 7. After 7 days of cultivation, the diameter of the pathogen growth suppression zone was measured in two mutually perpendicular directions.

3. Results and discussion

The results of the study of the antagonistic properties of isolates are presented in table 1.

Table 1. Antagonistic activity of isolates against pathogenic bacterial strains (t=37°C).

| Experienced isolates | Pathogenic bacterial | Escherichia coli | Enterococcus faecalis | Pseudomonas aeruginosa | Staphylococcus aureus |
|----------------------|----------------------|-----------------|----------------------|----------------------|----------------------|
| 1                    |                      | 1,26±0,07       | 1,28±0,07            | 1,31±0,08            | 1,30±0,08            |
| 2                    |                      | 1,21±0,06       | 1,26±0,07            | 1,22±0,07            | 1,21±0,06            |
| 3                    |                      | 1,38±0,07       | 1,32±0,08            | 1,30±0,08            | 1,35±0,08            |
| 4                    |                      | 1,35±0,08       | 1,33±0,07            | 1,30±0,08            | 1,28±0,07            |
Table 1 shows that the most pronounced antagonistic activity is shown by isolates №9, 16, 20, 21 and 23.

According to literature data, it is known that during the preparation of silage, the temperature inside the herbal mixture changes from 30 to 45°C. Therefore, different isolates can change their antagonistic properties when the ambient temperature changes. Isolates that showed the most pronounced results (from table 1) were selected for further experiments.

The antagonistic activity of isolates against pathogenic bacterial strains at a temperature of 30°C is shown in table 2.

**Table 2.** Antagonistic activity of isolates against pathogenic bacterial strains at a temperature of 30°C.
From table 2 it is seen that with decreasing temperature up to 30°C big changes in antagonistic activity of all investigated isolates was not observed, but a tendency to its decrease.

The antagonistic activity of isolates against pathogenic bacterial strains at a medium temperature of 45°C is shown in table 3.

**Table 3.** Antagonistic activity of isolates against pathogenic bacterial strains at 45°C.

| Experienced isolates | Pathogenic bacterial | Escherichia coli | Enterococcus faecalis | Pseudomonas aeruginosa | Staphylococcus aureus |
|----------------------|----------------------|------------------|-----------------------|------------------------|-----------------------|
| 9                    | 1.75±0.10            | 1.73±0.10        | 1.71±0.09             | 1.76±0.11              |
| 16                   | 1.77±0.11            | 1.75±0.10        | 1.72±0.09             | 1.74±0.10              |
| 20                   | 1.82±0.11            | 1.82±0.11        | 1.79±0.10             | 1.78±0.11              |
| 21                   | 1.90±0.12            | 1.91±0.12        | 1.78±0.10             | 1.84±0.12              |
| 23                   | 1.82±0.11            | 1.84±0.11        | 1.72±0.11             | 1.75±0.11              |

When the temperature of the medium increases to 45°C, more significant changes in the antagonistic activity of various isolates are observed. The activity of isolate 20 does not decrease, so it is resistant to high temperature. Isolate №21 has a significant increase in antagonistic activity, it is probably more resistant to high temperatures and some biochemical enzymatic processes are stimulated, leading to increased antagonistic properties. And in isolates 9, 16 and 23, on the contrary, it decreases, possibly due to the fact that the increased temperature causes structural and biochemical changes in the cell of the microorganism. Similarly, isolated isolates № 9, 16, 23, 21, and 15 had high antagonistic activity against micromycetes that contaminate plant-based feed.

**4. Conclusion**

Thus, the conducted research indicates that it is possible to consider promising for further work on the selection of strains for use in the development of drugs to protect feed from pathogens of bacterial and fungal nature during storage when the ambient temperature increases.

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