Paramecium caudatum abundance dynamics on various nutrient substrates consisting of Bacillus subtilis strains and their influence on morphophysiological indicators

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Abstract. According to the results of the study, data on the dynamics of the number of ciliates on various substrates were obtained. The maximum abundance, after 14 days of cultivation, was detected on a substrate from Bacillus subtilis of a natural strain (88 pcs / ml), on a substrate of Bacillus subtilis 2335 the abundance was slightly lower (81 pcs / ml), the minimum abundance was recorded on a substrate of Sacharamices ellipsodes (68 pcs / ml) A difference in morphophysiological characteristics was revealed, using the example of the qualitative and quantitative composition of digestive vacuoles. Indirect evidence of the participation of glycocalyx mechanisms in the choice of food by infusoria is given.

1. Introduction

Paramecium caudatum is an optimal object of hydrobiological research because it is easy to cultivate, it increases its number in a short time and is able to consume almost any bacterial cells, as well as microphytoplankton. In addition, paramecium is used as a test object for natural reservoirs biotesting [1-9].

In fish farming practice, P. caudatum is used as a standard feed for fish larvae 3–7 mm in size. However, the use of one P. caudatum for feeding fish is not effective, due to its low nutritional value, therefore, other organisms are used with it, for example: rotifers, microalgae, nematodes.

As a standard, a native strain of Bacillus subtilis grown on meat-peptide agar is used to survive P. caudatum.

Based on the above, the following purpose of the study was determined: to identify the dynamics of the number of Paramecium caudatum when feeding various strains and yeast cells; to assess the effect of various feed media on the morphophysiological parameters of the ciliates, as well as to establish the causes of functional changes, if any.

The expediency of enriching the culture has been revealed to use a specially derived strain of bacteria Bacillus subtilis 2335, which is part of the probiotic preparation Subalin (trade name SUB-PRO). This strain is distinguished by its bacteriostatic properties. Such antagonistic activity is manifested due to the fact that the culture of organisms releases specific substances, peptide (ribosomal synthesized) and non-peptide, whose antibacterial activity covers a wide list of gram-positive and gram-negative bacteria,
fungi and viruses [1]. In addition, there is information that some B. subtilis strains produce interferons. This determines the use of Bacillus subtilis 2335 as an active component of probiotic preparations.

2. Materials and methods
P. Caudatum was cultured according to the standard procedure [10], in glass crystallizers with a volume of 300 ml. at a temperature of 24-26 °C for 14 days.

All vessels were divided into three groups: the first two groups were grown on a substrate from yeast Sacharamices ellipsodes and bacteria of the natural strain Bacillus subtilis. S. ellipsodes was obtained by chipping on Guase No. 2 medium at 24 °C [8], and B. Subtilis on meat and peptone agar at the same temperature. The third group of ciliates was grown on a substrate of Bacillus subtilis 2335. In the first half of the vessels, by dilution, the concentration of microorganisms was brought to 1 * 10⁹ CFU / ml, in the second to 0.5 * 10⁹ CFU / ml. All experiments were performed in duplicate.

At the beginning of the experiment, 250 pieces of ciliates were added to the laboratory containers. Counting the number of ciliates was carried out in 1 ml, on 1, 3, 7, 10 and 12 days. To determine the quantitative composition and qualitative characteristics of the digestive vacuoles, microscopy was used, with an increase of 2000x.

3. Results and discussion
According to the cultivation results, the following data were obtained: the most rapid growth of the ciliates culture occurred on the natural B. Subtilis strain, at a concentration of 1 * 10⁹ CFU / ml, and the initial planting density of 1 individual per ml (hereinafter pcs / ml), on the 7th day the number of ciliates was 273 pcs / ml. Microscopic analysis showed that the maximum size of the ciliates reached 550 microns, they acquired an ovoid shape with a large number of digestive vacuoles filled with bacterial cells. Also, they were characterized by low mobility.

At a concentration of 0.5 * 10⁹ CFU / ml, that is, when diluting the initial concentration in half, the reproduction dynamics was lower, 110 pcs / ml. No morphological abnormalities were observed in these individuals. In general, they were similar to ciliates at a higher concentration of B. Subtilis (figure 1).

![Figure 1](image1.png)

**Figure 1.** P. caudatum reproduction dynamics of when feeding B. Subtilis natural, S. Ellipsodes: Blue colour - concentration 1 * 10⁹ CFU / ml, orange colour - concentration 0.5 * 10⁹ CFU / ml.

Feeding of ciliates with a strain of Bacillus subtilis 2335 cultivated at a concentration of 1 * 10⁹ CFU / ml and 0.5 * 10⁹ CFU / ml did not give results. Apparently, one of the possible factors hindering the development of the culture of ciliates could be a violation of the storage regimen of the drug or the expiration of its shelf life. In this regard, it was decided to increase the concentration of bacterial cells to values of 5 * 10⁹ CFU / ml and 10 * 10⁹ CFU / ml. In this case, after 7 days of cultivation, the number of ciliates reached 46 pcs / ml, in the second 88 pcs / ml (figure 2).

![Figure 2](image2.png)
Microscopic analysis did not show significant differences in morphology, with ciliates feeding on a natural strain. This may indicate that the B. subtilis 2335 bacterial strain is a suitable food item with high nutritional value and optimal biochemical composition.

The number of digestive vacuoles in the culture of ciliates on the substrate B. subtilis 2335 was quite indicative. So, it was possible to distinguish all types of digestive vacuoles: phagosome (vacuole - I), phagoacidosome (vacuole - II), phagolysosome (vacuole - II) (figure 3), and the direction of cyclosis (figure 3). Moreover, the diameter of phagosomes and phagolysosomes in different individuals varied from 15 to 24 μm (measurements were made in 20 individuals), which slightly exceeds the standard value (15 μm [3, 6]).

Feeding with yeast culture showed lower results in the rate of ciliates breeding. At a concentration of fungal cells of 1 * 10^9 CFU / ml, the number of ciliates was 75 pcs / ml on day 14 of the experiment. Halving the concentration of yeast (0.5 * 10^9 CFU / ml) led to a decrease in the maximum number of paramecium by 60%, up to 38 pcs / ml. The morphology of individuals was somewhat changed, compared with the natural type, the size of the ciliates ranged from 300-450 microns. Microscopic analysis showed a decrease in the length and width of the body, lengthening of the cilia, as well as a decrease in the number of digestive vacuoles (figure 4).
A higher mobility of the Paramecium in culture was observed. This feature can be explained by the fact that the genus of the ciliate Paramecium is not always able to recognize the nutritional value of the absorbed particles. So, in view of the small size and the presence of the second glucan layer of the cell wall of the yeast cells its ingestion occurs rapidly, but the Paramecium does not digest it, bringing back into the environment [3]. It is also worth to add that the selectivity of Paramecium in food, apparently, is carried out using the glycoproteins of the glycocalyx, it is not necessary to eliminate the contribution of ciliary activity. Most of them are immobilization [4] and surface antigens [5], which, in turn, may perform the receptor function. They are distributed throughout the body, representing glycoprotein membrane [4], thereby allowing the cell to respond to diverse environmental signals, to determine the chemical characteristics of food particles, and if necessary to initiate phagocytosis.

The digestive vacuoles at the ciliates, when fed with the S. ellipsodes yeast culture, were less distinguishable and their total number was less than the ciliates on the B. subtilis 2335 substrate. Despite this, it was possible to clearly determine the direction of cyclosis, and, consequently, all three types of digestive vacuoles (figure 3). According to the results of measurements in 20 individuals, the diameters of phagosomes and phagolysosomes were in the range from 13 to 18 μm, which is lower than the marginal values for the P. caudatum culture fed on B. subtilis 2335. Incomplete digestion of yeast cells was also observed.

Based on the above, it can be concluded that the ciliates grown on a pure culture of Bacillus subtilis 2335 cells are a more suitable forage object for fish larvae, due to their larger size and lower speed of movement. The presence of this culture in water can reduce the number of pathogenic bacteria, due to its antagonistic activity, which positively affects the growth of fry. It is this advantage that distinguishes the B. subtilis 2335 strain in comparison with the natural one. In turn, the natural strain shows the best results in the number of individuals per ml, since meat-peptide agar has a more balanced composition, which contributes to a faster reproduction of paramecium.

4. Conclusion
The results of the study revealed that all considered variants nutrient substrate allow to obtain enough high density of ciliates culture and use it for feeding fish larvae. Possible use of ciliates grown in culture, B. Subtilis 2335, for the prevention of bacterial fish diseases due to their antagonistic activity, in consequence of which decreases the number of pathogenic species in the water microbiocenosis.

The yeast biomass used as a forage substrate, changes the morphology of the protozoa and affects their physiological responses, inhibiting their reduced body size, the amount and composition of digestive vacuoles.

Figure 4. Digestive vacuoles of P. Caudatum, on a substrate of Sacharamices ellipsodes: Ph - phagosome; PhA - Phago-acidosome; PhL - Phagolysosome; Cyt - Cytopharynx; Cyc - Cyclosis.
Most reliable results of growth were obtained on natural strain, 88 PCs/ml, due to the optimal composition of the microbiological environment in which B. Subtilis were cultivated.

Qualitative and quantitative differences in the digestive process of P. caudatum, for example, the digestive vacuoles, are demonstrated. So, when feeding Paramecium S. Ellipsodes, size and number of phagosomes and fagolysosomes was lower than when feeding B. Subtilis. The phagoacidosome was almost not distinguishable.

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