Red yeast *Rhodotorula benthica* – substitute feed base for echinoderms in factory cultivation

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Abstract. Now decrease press of the fishery on natural populations of hydrobionts and restoration of their number due to artificial reproduction is actual. At artificial cultivation important properly to choose the right feed. To develop the feeding technology, work was carried out with larvae and juveniles of sea cucumber and gray sea urchin in the conditions of the mini-plant of the Scientific and Production Department of Mariculture of the Far Eastern State Technical Fisheries University located in Severnaya Bay (Slavyansky Gulf, the Sea of Japan) and Troitsa Bay (Vladimir Gulf, the Sea of Japan). Experimental studies have shown the possibility of using the sea yeast Rhodotorula benthica as either the main feed or as a feed additive. Practical recommendations have been developed for the use of Rhodotorula benthica yeast as a substitute feed base for growing larvae and juveniles of Japanese sea cucumber and gray sea urchin. The results of the work will serve as a basis for the development of biotechnology for feeding at echinoderm breeding farms and will be useful to the organizations, whose duties include rational and competent progress of marine economic territories.

1 Introduction

Currently, the urgent task is artificial reproduction of echinoderms to preserve their numbers in natural populations. Japanese sea cucumber (Apostichopus japonicus) is one of the most expensive and commercially successful object on the market. An effective way of reinforcing the natural accumulation of holothuria and the commercial product output is the development and implementation of the juveniles rearing technologies at the industrial facilities. In artificial cultivation, it is important to choose the right biotechnology of feeding the cultivated object. [1,2] At the present time regardless of all the recommendations and standards developed for all stages of the technological process, the problems of selecting a more stable fodder base (diet) for the larvae and settled juveniles remain unsolved; the diseases that occur at any development stage and the uncontrollable biotic factors of the media still can cause the death of the entire reared colony of animals [3].

Nowadays, it is important to optimize technological processes at enterprises for the cultivation of echinoderms by using alternative feed bases or additives for juveniles and larvae.

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The objective of this study is to develop practical recommendations on the use of the yeast *Rhodotorula benthica* as a substitute feed base for growing larvae and juveniles of the Japanese sea cucumber and gray sea urchin.

2 Research materials and methods

To develop practical recommendations for feeding the research work was carried out with larvae and juveniles of sea cucumber and gray sea urchin in the conditions of the mini-plant of the Scientific and Production Department of Mariculture of the Far Eastern State Technical Fisheries University located in Severnaya Bay (Slavyansky Gulf, the Sea of Japan) and Troitsa Bay (Vladimir Gulf, the Sea of Japan).

In the course of experimental studies, the red yeast *Rhodotorula bentica* was used as a feed base for larvae, and for settled juveniles as a feed additive. The method of laboratory cultivation of *Rhodotorula bentica* yeast biomass was described by us in a previously published work [4].

3 Research results

According to literature data, in China, sea yeast of the genera *Rhodotorula* and *Debaryomyces* is successfully used as a probiotic feed additive for cultivated aquatic organisms [5-7]. Previously, we carried out a preliminary study, during which no significant differences were found in the time and speed of the onset of the stages of larval development and survival of the sea cucumber, both when the sea yeast *Rhodotorula benthica* was added to it as food, and when fed with the microalgae *Chaetoceros muelleri* and *Dunaliella salina* [3]. Therefore, repeated studies with sea cucumber were carried out and it was decided to conduct experimental feeding of larvae and juveniles of the gray sea urchin. Before using red yeast as feed, the viability of yeast cells is preliminarily determined by the method of staining with methylene blue, followed by microscopy of the diluted yeast suspension. More than 80% of the cells need to be viable.

To provide food for 8.2 million larvae of sea cucumber at the “early auricularia” stage (subsequently 1 million settled juveniles), about 4 kg of dry biomass of the *R. benthica* yeast is needed. The concentration of cells in 1 g of dry matter can reach 80 billion cells.

The number and frequency of feeding were defined experimentally by examining the stomachs of the larvae, after feeding. The average stocking density of larvae at the "early auricularia" stage and the concentration of the introduced feed in Severnaya Bay and Troitsa Bay are presented in tables 1 and 2.

Table 1. Average larvae stocking density and the amount of the introduced food base in Severnaya Bay

Stocking density (individuals/ml)	Feed concentration	
	Rhodotorula benthica (cells/ml)	Amount of dry biomass (g)
2	20 000	0.5
4	40 000	1
1	10 000	0.25
3	30 000	0.75
1	10 000	0.25
0.4	10 000	0.25
0.5	10 000	0.25
0.7	10 000	0.25

Table 2. Average larvae stocking density and the amount of the introduced food base in Troitsa Bay

Stocking density (individuals/ml)	Feed concentration	
	Rhodotorula benthica (cells/ml)	Amount of dry biomass (g)
3.5	70 000	1.75
2	40 000	1

Recommendations for the feeding regime with red yeast for the larvae of the Far Eastern sea cucumber and gray sea urchin at different stages of development are presented in Table 3.

The previously weighed dry mass of yeast, for each pool with larvae, must be dissolved in a sufficient amount of purified seawater (the ratio of 250 ml of water and yeast according to Tables 1-4) and mixed. The finished suspension is added to production containers. Feeding should be done before changing water in the pools. In the first weeks after settling of juveniles (individual sizes are less than 0.1 cm), feeding was carried out with a mixture of red yeast and a filtered homogenate of attached diatoms algae. As the size of juveniles increases - 0.5 mm or more, detritus or compound feed is added to the diet. Recommendations for the feeding regime of red yeast for the larvae of the Far Eastern sea cucumber and gray sea urchin at different stages of development are presented in Tables 3, 4.

Table 3. Feeding regime of Apostichopus japonicus and calculation of the amount of *R. benthica* yeast biomass

object	**Development stages**	**Stocking density (ind./ml)**	**Added feed concentration (cells/ml)**	**Feeding frequency (times/day)**	**Amount of dry biomass (g)**	**Usage**
Apostichopus japonicus	Early auricularia	1	20 000	3-4	0.5	As feed base
	Medium auricularia					
	Late auricularia		40 000 – 60 000		1-1.5	
	Deliolaria					
	Pentactula					
	Settled juveniles		60 000	2-3	1.5	As feed additive
Strongylocentrotus intermedius	Pluteus I	1	30 000	3-4	0.75	As feed base
	Pluteus II		40 000 – 50 000		1-1.25	
	Pluteus III					
	Settled juveniles		50 000 – 60 000	2-3	1.25-1.5	As feed additive

Table 4. Feeding regime for R. benthica as feed additive (per 1 million of settled juveniles)

Object	**Juveniles size**	**Feed**	**Concentration (g)**	**Feed regime**
Apostichopus japonicus	less than 0.1 cm	homogenate of macrophytes	30	2-3 times/day
		R. benthica	1.5	
	more	homogenate of macrophytes	100	

	than 0.5 cm	R. benthica	1.5	
	more than 1 cm	homogenate of macrophytes	100	
		R. benthica	1.5	
		compound foodstuff	5-20	
Strongylocentrotus intermedius	less than 0.1 cm	homogenate of macrophytes	50	2-3 times/day
		R. benthica	1.25-1.5	
	0.5-1 cm	homogenate of macrophytes	30	
		R. benthica	1.25-1.5	
		Powder laminaria	50	

4 Conclusions

The data obtained on the use of red yeast as a substitute feed base are preliminary and require further research. After reception of base technology are measured to develop procedures of enrichment of this forage various bioactive additives. The forage from red yeast is stipulated for larval stages of a trepang and a gray sea urchin, but in the further we wish to create the whole ruler of forages – and for other stages of progress as echinoderms, and other traditional objects of mariculture.

The results of the work will serve as a basis for the development of biotechnology for feeding at echinoderm breeding farms, will be useful to the organizations, whose duties include rational and competent progress of marine economic territories. The use of a traditional food resource based on microalgae is sometimes problematic; therefore, it seems appropriate to use the sea yeast *Rhodotorula benthica* as either the main food or as a feed additive.

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