ANIMAL HUSBANDRY

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RAINBOW TROUT: BIOTECHNOLOGICAL AND GENETIC ASPECTS OF FARMING AND BREEDING

Review article

Abstract

An important problem of fish farming in is the accumulation of organic matter and ammonia in the water reservoir. Breeding work in fish populations is carried out using both mass selection and individual selection in families (family selection). Data on the genetic characteristics of trout, such as natural polyploidy and high genetic variability at quantitative traits, are presented. The purpose of the review is to consider the issues of modern technology for fish breeding to show the fesibility of bacterial water purification during its recirculation in water systems, to summarize the latest achievements of genetics and biotechnology that have found application in industrial fish farming in the country. The review considers biotechnological solutions to the problem of the accumulation of organic substances and ammonia in water by fish breeding and selection of effective bacterial strains that make it possible to utilize these harmful substances, thus, reducing their concentration in water to an acceptable level. In this regard, the *Dyadobacter* sp. and *Janthinobacterium* sp., which, when used in combination, provide a comfortable environment for growing *Rainbow Trout*. At the same time, the concentration of ammonia in water declines from 13.2 ± 2.1 μg/ml to 8.8 ± 1.8 μg/ml, which meets the normative values. Thus, the review presents a detailed picture of the biological characteristics of trout fish, showing their potential for further genetic, breeding, and biotechnological research.

Keywords: bacterial strains, *Rainbow Trout,* ammonia accumulation, fish breeding, aquaculture.

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РАДУЖНАЯ ФОРЕЛЬ: БИОТЕХНОЛОГИЧЕСКИЕ И ГЕНЕТИЧЕСКИЕ АСПЕКТЫ ВЫРАЩИВАНИЯ И СЕЛЕКЦИИ

Обзорная статья

Аннотация

Важной проблемой рыбоводства в узв является накопление в водоёме органических веществ и аммиака. Селекционная работа в популяциях рыб проводится как с использованием массового отбора, так и с индивидуальным отбором в семьях (семейная селекция). Представлены данные о генетических особенностях форели, такие как природная полиплоидность и высокая генетическая вариабельность по количественным признакам. Цель обзора - рассмотреть вопросы современной технологии разведения и селекции рыбы, показать возможности бактериальной очистки воды при ее рециркуляции в замкнутых системах, суммировать последние достижения генетики и биотехнологии, которые нашли применение в промышленном рыбоводстве страны. В обзоре рассматриваются биотехнологические решения проблемы накопления в воде органических веществ и аммиака в виде селекции и отбора эффективных бактериальных штаммов, позволяющих утилизировать эти вредные вещества, снизив их концентрацию в воде до приемлемого уровня. В этом плане эффективными оказались штаммы *Dyadobacter* sp. и J*anthinobacterium* sp., которые при комбинированном использовании обеспечивают комфортные условия для выращивания радужной форели. Концентрация аммиака в воде при этом снижается с 13.2 ± 2.1 мкг/мл до 8.8 ± 1.8 мкг/мл, что соответствует нормативным показателям. Таким образом, обзор представляет развернутую картину биологических особенностей

форелевых рыб, показывающий их привлекательность для дальнейших генетических, селекционных и биотехнологических исследований.

Ключевые слова: штаммы бактерий, радужная форель, накопление аммиака, разведение рыбы, аквакультура.

1. Methods of breeding *Rainbow Trout*

The gradual depletion of natural fish stocks in natural reservoirs due to active and often uncontrolled fishing dictates the need to expand aquaculture production and use new biotechnological approaches in fish breeding. In particular, now a lot of attention is paid to industrial breeding new industrial forms of *Rainbow Trout*, because nowadays obtaining quality products with dietary properties is a market requirement. From this point of view, trout farming is one of the most promising areas of fish farming in aquaculture. *Rainbow Trout* (*Oncorhynchus mykiss*) has a good growth rate, high dietary properties of meat and high fertility, which makes it one of the main objects in aquaculture [1], [2].

One of the leading fish breeding and genetic centers in Russia is located at the village of Ropsha, Leningrad Region. For many years, this experimental farm has been engaged in breeding and creating new breeding forms of fish, primarily of the salmon family. Several new breeds have been generated, such as Rofor and Rostal. A new genetic variant, the Golden Trout, is now undergoing evaluation and approval. The formation of this variant at initial first generations was carried out by methods of mass selection with the use, in some cases, of elements of family breeding [3]. The use of the method of matching pairs of individual females and males according to the selected type of color led to the formation of desirable phenotypes in the offspring and the consolidation of this trait. A characteristic feature of trout fish is variability by many quantitative traits, as well as a high frequency of abnormal forms of fry. Raising *Rainbow Trout* is carried out in cages, pools and recirculating aquaculture systems (RAS) [4].

Along with the benefits, cage farming has its drawbacks. The main one is eutrophication - pollution of a reservoir with organic substances. Dense fish stocking and intensive feeding led to progressive eutrophication of the water reservoir. To prevent this process, the area of cages in the reservoir should not exceed 0.1% of the area of the entire reservoir. In pools of a limited area, young trout are grown in the amount of 500-1000 individuals (Fig. 1).

Figure 1 – Trout growing in pool

Farming of trout in RAS is a very promising direction in aquaculture. This ensures control over the temperature and quality of water and optimizes fish production, improves health, prevents fish from escaping the facilities and the introduction of pathogenic microbes, regulates the incoming (clean water) and outgoing (waste water) water flows. With increasingly stringent environmental requirements and decreasing groundwater levels in most areas, recirculating water systems are the preferred systems for use in aquaculture.

Rainbow Trout grows quite well under RAS conditions. During the year the fish grows to a weight of 900-1400 grams. The recirculating water system provides optimal conditions: water temperature 13-17°C, saturation with dissolved oxygen, dissolved CO_2 <25 mg/L, nitrite nitrogen <0.3 mg/L, alkaline water and hydraulic system containers provide self-cleaning and optimal speed of movement of fish. In growing tanks, the volume of water is changed once every 15-30 minutes. Constant lighting is maintained throughout the day, mechanical feeders with timers are used, and thus, fish are fed in equal portions 8-24 times a day, that is, every 1-3 hours. This feeding scheme allows maintaining constant water quality in the RAS, biological respiration, oxygen demand and pollution levels. *Rainbow Trout* feeds aggressively, so slowly sinking feed is used, which is spread by rotating discs. This helps to reduce competition for food among fish. In flowing pools with good water quality, the growth of trout depends on the water temperature. In the autumn-winter-spring period, growth slows down due to low temperatures, this period is about six months, and trout grows best in summer. Under RAS conditions, at the optimum water temperature, trout grows all year round. Higher temperatures, as well as lower temperatures, negatively affect the development of fish [5].

Trout growth in RAS, compared to other cultivation methods, accelerates significantly: from fry (weight about 1 g) to marketable weight (250-300 g), trout is grown in less than six months. At the same time, there is no dependence on the season.

An important advantage of RAS is its ability to prevent diseases. In contrast, in the ordinary pool from the surface runoff, infections and chemical pollutions can get into the water.

2. Obtaining gametes for fertilization

In the nursery where spawning is carried out, the best producers are selected and separately transferred to small pools with a stocking density of 25-30 individuals/m². The transferred fish is monitored daily and, as the eggs of the females mature, they are sifted out. The readiness of caviar is judged as follows: when pressing on the abdomen, mature roes easily come out of the anus. A thorough quality control of the obtained reproductive products - caviar and sperm - is carried out. For example, whitened roes are dead and are excluded from further use (Fig. 2).

Figure 2 – Whitened (dead) and normal alive roes

3. Genetic aspects of trout farming

Trout is a well-studied fish species in terms of its biology and genetic characteristics. In particular, the tetrapolidity of trout is well documented [6]; the phylogeny of various species and subspecies was studied using microsatellite DNA [7]. A wide public response was caused by the appearance on the market of transgenic salmon, which was obtained by introduction of growth hormone gene of Chinook salmon, which led to an increased synthesis of this hormone in the trout liver. The control of gene expression was carried out by a promoter taken from the eelpout antifreeze protein gene. The accumulation of mass in transgenic salmon, on average, was 2 times faster than in normal non-transgenic individuals.

In order to get as many females as possible, the sex is reversed, that is, unisex original individuals are obtained. Reversed males mature later and therefore grow faster before maturation; more efficiently assimilate feed and produce caviar, an expensive food product. Sex is determined genetically and hormonally, more often genetically. Females carry the XX sex chromosomes (all gametes carry an X chromosome), males carry the XY sex chromosomes (X and Y gametes). When gametes X and X merge, a female is obtained, gametes X and Y produce a male. The presence of the X chromosome leads to the synthesis of estrogen - the female sex hormone, the development of the body follows the female path. The presence of the Y chromosome leads to the synthesis of androgens - male sex hormones, the development of the body follows the male path. Both hormones are present in the fish organism, but one of them prevails, determining the development of the organism and, first of all, the gonads. Therefore, if a larva with chromosomes XX is treated with androgens, its development will follow the male path, and the gonads will produce spermatozoa containing only the X chromosome. This procedure is called gender reversal.

Over the years, researchers have paid considerable attention to DNA technologies in breeding, in particular, the use of allelic variants of individual genes in animals associated with productive traits [8], [9], [10], identification of bacterial strains [11] and genome-wide analysis of polymorphic sites in fish [12]. Another interesting fish breeding approach was the use of triploids. Scientists have long noticed that in salmonids there are triploid and tetrapolid individuals, ploidy mosaics, and there are also duplicated individual genes in the genome [6]. Experimentally, tripolides can be obtained by intraperitoneal transplantation of immature germ cells of spermatogonia into embryos, followed by their growth to an adult state. Eggs and sperm are taken from the obtained triploid individuals, fertilization and fry rearing are carried out. The offspring consists of normal diploids, triploids and tetraplolids. In some cases, by analogy with tetraplolid plants, the fish showed faster growth in comparison with ordinary diploid individuals.

4. Biotechnological aspects of trout farming

In the process of protein catabolism in fish, ammonia is produced, which is removed from the blood through the gills. Decreased growth, tissue erosion (kidney, gills and skin) and degeneration, suppression of immunity and high mortality of fish are associated with the accumulation of large amounts of ammonia in aquatic systems.

Nitrification is a microbial process that can be used to reduce or eliminate unwanted nitrogen in recirculating aquaculture systems and thus make water recycling possible on a larger scale. Nitrification can take two forms: with autotrophic and heterotrophic bacteria, these bacteria can sometimes function in association. It was found that the autotrophic removal of ammonium and nitrite occurs faster in the presence of heterotrophic strains.

In a recirculating aquaculturer system, biological filters are commonly used to reduce ammonia in water. The biofilter used for RAS is an important piece of equipment. Its purpose is to purify water from harmful organic and inorganic substances. A mechanical filter cannot remove organic compounds and substances of a mineral nature (phosphates, nitrogen, etc.). Phosphates do not have toxic properties for fish. Nitrogen in the form of ammonia (NH_3) is a toxic substance that must be converted into harmless nitrate on a biofilter. The transformation of organic compounds and ammonia is possible using biotechnological processes on biofilters based on the use of active bacterial strains. Heterotrophic bacteria are able to assimilate oxygen to oxidize organic compounds to CO_2. Strains of nitrifying microorganisms convert free ammonia into nitrite, followed by its conversion into nitrate. The efficiency of this process is a function of the water temperature, the pH level in the water, and the presence of water inhibitors surressing bacteria activity.

Ammonia is present there in two forms: non-ionized and ionized. In terms of toxicity, the non-ionized form is important for aquatic animals. Since both groups of heterotrophic and autotrophic bacteria are capable of nitrification, there is competition for nitrification between these two groups in the recirculation system. Heterotrophic bacteria gain the upper hand in nitrification when the ratio of dissolved organic carbon to nitrogen increases. This ratio can be increased by the activity of heterotrophic bacteria, the accumulation of dead bacteria, and the assimilation of ammonia into microbial biomass. It was found that with an increase in this ratio, heterotrophic nitrifiers can have 2-3 times higher activity compared to autotrophic bacteria. Heterotrophic *Dyadobacter* sp. and *Janthinobacterium* sp. from ecological isolates are used to remove non-ionized ammonia and nitrite in the trout cultivation system. In addition to these microorganisms, *Pseudomonas migulae* has also found application [13].

In nature, microorganisms use a variety of strategies to combat nitrogen starvation. *Dyadobacter* sp. can even grow on Bourke's N-deficient medium due to its ability to fix atmospheric N_2. Despite a slight decrease in activity at 15°C, this species can effectively reduce the ammonium concentration in aquaculture. To date, various strains of bacteria of the genus *Janthinobacterium* have been isolated from many water and soil sources [14], [15]. It is known that representatives of this genus decompose nitrite at low temperatures [1]. Well known also antifungal and antibacterial activity of bacteria of the genus *Janthinobacterium*. These microbial properties are very important in aquaculture systems where opportunistic microorganisms are a constant threat to fish. On the other hand, there are indications that under certain conditions the bacteria *Janthinobacterium* sp. may be pathogenic against trout [16].

A heterotrophic microbial complex of two strains of bacteria *Dyadobacter* sp. and *Janthinobacterium* sp. (HAN) has established itself as an effective way to remove ammonium and nitrite. Compared with the standard culture setup, the addition of the HAN complex had a clear beneficial effect on maintaining the level of non-ionized ammonia and nitrite below the established standards ($P < 0.05$). The use of the selected microbial complex leads to a significant improvement in the aquaculture ecosystem (table) [17].

Table 1 – The effect of heterotrophic ammonium and nitrite removal set (HAN) on concentration of un-ionized ammonia, nitrite, nitrate, dissolved oxygen, pH, and temperature in a *Rainbow Trout* ($n = 50$) breeding system after 9 days [17]

Water properties	Groups		
	HAN	Control	Recommended standards
NH_3-N, µg/L	8.8 ± 1.8*	13.2 ± 2.1*	12.5
NO_2-N, µg/L	62 ± 11	31 ± 15a	1000
NO_3-N, µg/L	15.61 ± 5.36	13.13 ± 4.12	<400
Temperature, °C	14.15 ± 1.17	14.13 ± 1.17	<16
pH	6.85 ± 0.09	6.25 ± 0.17	6.5 – 8.5
Dissolved O_2 (mg/L)	8.65 ± 2.12	8.51 ± 3.19	>6.0

In these aqua systems, biofilters with microorganisms convert harmful components such as ammonium into nitrites and nitrates. Suitable *Janthinobacterium* sp. demonstrates impressive heterotrophic nitrification efficiency with significant nitrite-removing activity [17], the growth temperature for most aerobic heterotrophic bacteria used in biofilters is 28°C, while the optimum temperature for trout growing is around 15°C. Therefore, there is a problem of finding microorganisms that can grow and effectively remove ammonium at lower temperatures required for aquaculture of trout [13], [18]. Given the large genetic diversity of soil and water microflora, it is possible to identify and use strains that can grow at low temperatures. Identification of bacteria is carried out by sequencing 16S rRNA fragments with subsequent comparison of nucleotide sequences with known NCBI databases. Selection of bacterial species and strains is carried out by growing cultures at low temperatures with the identification of the most actively growing bacteria. According to sequencing data, it was found that the isolated strain numbered 100 was 95% identical to the non-pathogenic bacterium *Janthinobacterium svalbardensis* (KR 085903.1). Therefore, this strain was selected for further analysis as the optimal nitrite remover. This microorganism can reduce nitrite to nitric oxide using nitrite reductase proteins.

As a result of the activity of *Dyadobacter* sp. ammonium is expected to be removed by converting it to nitrite. As a result, nitrite build-up can be expected, which is detrimental to trout culture. However, nitrite accumulated as a product of *Dyadobacter* sp. is effectively eliminated by *Janthinobacterium* sp. in the HAN microbial complex, bringing the concentration of nitrite to 62 ± 11 µg/L, which is below the limit value of the range (1000 µg/L) (table). Water quality characteristics such as dissolved oxygen and temperature did not show any significant differences between groups.

Ammonium and nitrite levels in *Rainbow Trout* aquaculture can be reduced in the presence of *Dyadobacter* sp. and *Janthinobacterium* sp. *Dyadobacter* sp. (strain no 68) and *Janthinobacterium* sp. no. 100 was applied to the trout breeding system as a mixed culture [17]. As expected, the results obtained showed that the concentration of non-ionized ammonia in the untreated negative control group was higher than the recommended limits for trout growing. The level of non-ionized ammonia in the HAN group (8.8 ± 1.8 μg /L) was significantly lower than in the control (13.2 ± 2.1). This indicated that the bacterial complex was efficient in removing non-ionized ammonia in an aquaculture system.

5. Conclusion

Recirculated aquaculture systems are a modern method of trout fish farming, providing reliable temperature control and reliable regulation of water quality. This gives rize to greater efficiency of trout farming, improves fish health by reducing the concentration of harmful organic and inorganic substances, increases productive qualities and prevents pathogenic microflora from entering into the water. Under modern conditions of aquaculture, the methods of genetics and biotechnology are of particular importance, offering strains of highly effective heterotrophic bacteria for use on biofilters, which make it possible to reduce the concentration of ammonium, nitrates and nitrites in recirculated water to acceptable level.

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Conflict of Interest

None declared.

Конфликт интересов

Не указан.

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