Cytogenetic homeostasis of African catfish in high-tech industrial aquaculture

E V Spirina, E M Romanova, V N Lyubomirova, V V Romanov and M E Mukhitova

Ulyanovsk State Agrarian University named after P A Stolypin, 1, Novy Venets Blvd., 432017, Ulyanovsk, Russian Federation

E-mail: vvr-emr@yandex.ru

Abstract. Sharptooth catfish (Clarias gariepinus) is a prospective object of aquaculture. It is fast-growing species, which growth period from larva to commodity fish makes only 6 months. Its meat is rich with essential fatty acids (omega-3) and satisfies modern requirements to healthy food. One of efficient ways to prevent stressful factors of fish cultivation in the conditions of industrial aquaculture is the use of complete feed. Biologically active agents, including probiotics, are important in the balance of feed macronutrients for cultivation of physiologically competent fish. Sporotermin is among effective probiotics. The purpose of the study was to define cytogenetic homeostasis of African sharptooth catfish (Clarias gariepinus) at the introduction of probiotic Sporotermin into feed. The study was conducted during cultivation of catfish in hi-tech industrial aquaculture. The cytogenetic homeostasis was assessed via the micronucleus test. The micronucleus test on fish is used to assess the pollution of their habitat. The study also revealed differences in the frequency of micronuclei and their types between group of fish growing with and without probiotic Sporotermin. Sporotermin decreases cytogenetic homeostasis disorders. Thus, the number of cells with (a) micronuclei decreases from 0.4% to 0.075%. There are no cells with micronuclei of (b), (c), (d-I), (d-II) and (e) types against the background of application of Sporotermin as a feed additive. The study was supported by the Russian Foundation for Basic Research, grant No. 18-016-000127.

1. Introduction
At present there is the growing demand for products of aquaculture, which main objective is to supply a wide range of fresh fish-breeding products not only in terms of species diversity, but also at affordable prices to the population with different income level. Besides, the population is concerned with health and safety of food ensuring the development of this branch of agriculture. Modern aquaculture cultivation technologies allow reducing seasonality of production and increasing the level of its automation thus expanding geographical boundaries of aquaculture objects to get environmentally friendly and virus-free products, as well as ensuring the import substitution.

Sharptooth catfish (Clarias gariepinus) is a prospective object of aquaculture. It is fast-growing species, which growth period from larva to commodity fish makes only 6 months. Its meat is rich with essential fatty acids (omega-3) and satisfies modern requirements to healthy food. Besides, biological features of sharptooth catfish save a lot of energy for optimization of environmental parameters during industrial cultivation methods. It also has high digestion efficiency [1]. The Sharptooth catfish is an
object of commodity fish breeding in China, Thailand, the Philippines. In Russia it is grown in Lipetsk, Kursk, Ryazan, Krasnodar Krai.

It is important to consider certain stressful factors in the cultivation of sharptooth catfish: transportation, fishing, transfer, density, compound feeds, medicines, planting stock, fish-breeding stock and equipment. One of efficient ways to prevent stressful factors of fish cultivation in the conditions of industrial aquaculture is the use of complete feed. Biologically active agents, including probiotics, are important in the balance of feed macronutrients for cultivation of physiologically competent fish [2]. Probiotic Sporotermin is among effective probiotics. Sporotermin transforms vegetative cells to spores under adverse conditions, which can last for a long time until reaching favorable conditions for growth. The spores can sustain acid attacks, heating over 100°C, freezing and radiation exposure. When getting to intestines into the alkaline environment, it creates the stimulus for rapid growth of bacteria colonies and their transition to active vegetative form. These properties ensure broad industrial use, after which spore-forming bacteria maintain their properties. Due to its feature the use of Sporotermin as part of compound feed at cultivation of sharptooth catfish allows reducing the level of opportunist microorganisms causing infectious diseases of fish: Staphylococcus aureus, Escherichia coli, Salmonella enteritidis, Salmonella sp., Salmonella choleraesuis, Pseudomonas aeruginosa, Proteus vulgaris, Candida albicans, Klebsiella pneumonia, Citrobacter freundii, Morganella morganii, Yersinia enterocolitica, Shigella sonnei, Enterobacter agglomerans, etc. [3].

One of the indicators to assess the condition of an organism is cytogenetic homeostasis [4] expressed by its ability to maintain karyotype. Cytogenetic homeostasis can be characterized via the micronucleus test that counts the number of cells with micronuclei [5]. Micronuclei are mainly formed from chromosomal material deprived of centromere through the formation of aberrations of chromosomes and therefore lagging behind the total number of disjunctive chromosomes at the anaphase stage. During mitosis this material only gets to one daughter cell and forms one or several small nuclei – the so-called micronuclei. Micronuclei mainly consist of acentric fragments, which was shown through DNA measurement [5, 6]. They can also be formed by the whole chromosome as a result of non-divergence caused by the defect of a spindle apparatus. Micronuclei can be found in cells of any proliferating tissue. The processes forming micronuclei undoubtedly demonstrate the decrease of cell viability thus serving a marker of their non-stability.

The micronucleus test is widely used to assess the effect of environmental pollutants on living organisms. The micronucleus test on laboratory animals became one of the most practical and short-term cytogenetic tests in toxigenetics [6-8, 10].

Besides, the analysis of micronuclei is of the utmost interest since it is relatively simple, quick and cheap. The advantages of the micronucleus test include not only quickness, but also independence from the karyotype of the studied species sometimes containing a large number of small low-observable chromosomes. Besides, the test can be held in tissues with low mitotic activity. The revealed correlation between the micronucleus test and the chromosomal aberration test allows considering the micronucleus test a good indicator of influence of various chemical agents [11]. At present, it is widely used for screening and monitoring of mutagens of different nature, including mutagenic impact of the environment [7, 9].

The purpose of the study was to define cytogenetic homeostasis of African sharptooth catfish (Clarias gariepinus) at the introduction of probiotic Sporotermin in high-tech industrial aquaculture.

2. Materials and Methods

African sharptooth catfish grown in the laboratory of the Department of Biology, Veterinary Genetics, Parasitology and Ecology of Ulyanovsk State Agrarian University was the material for the study. For the experiment the juveniles of sharptooth catfish were split into 2 experimental groups of 50 species
each. The groups were placed into autonomous 300-liter containers. Water temperature in all pools was similar – 26°C, oxygen content in water was at the level of 4 mg/l. Feeding of juveniles and maintenance of hydrochemical indicators of water quality was performed in a standard manner.

The peripheral blood of African sharptooth catfish was taken antemortem from a tail vein of species without visible external damages. The sample volume made 10 species of a group. The blood smears were dyed with acetic orcein. The agents were analyzed via microscope Mikromer 2 (3-20) at magnification of 2500x. On dyed agents we counted ≈2 thousand normal erythrocytes from each fish thus fixing (in addition to the quantity of normal erythrocytes) the number of cells with micronuclei. Micronuclei were typified according to the following scheme (Figure 1): “standard” micronuclei (a), “attached” (b), “connected to nucleus via chromatin fiber” (c), unshaped nuclear material in the form of sticks (d-I) or balls (d-II), quite big roundish nuclear material (e) [5]. The total number of cells with micronuclei was used to compare different samples. The statistical analysis of frequencies and comparison of shares of abnormal cells (i.e. cells with micronuclei) in different samples was carried out using t-criterion after φ-transformation of frequencies according to recommendations.

Figure 1. Schematic view of micronuclei types.

3. Results
The micronucleus test, calculation of erythrocytes with micronuclei were made to assess cytogenetic homeostasis of African sharptooth catfish grown with and without Sporotermin.

The average frequency of erythrocytes with micronuclei is presented in Table 1.

| Experiment         | Number of cells with micronuclei |
|--------------------|----------------------------------|
| Control            | 13.5                             |
| Sporotermin        | 1.5                              |

The study of peripheral blood samples of African sharptooth catfish showed the highest frequency of the cells with micronuclei of (a) type, then cells with micronuclei of (b) and (d-I) types (Table 2).

It shall be noted that almost each fish grown without Sporotermin had cells with micronuclei of any of the above types.

Table 2. Share of cells with micronuclei of different types

| Experiment       | Cells with micronuclei, % | Cells with different types of micronuclei, % |
|------------------|--------------------------|---------------------------------------------|
|                  | a    | b    | c    | d-I  | d-II | e    |
| Control          | 0.9  | 0.4  | 0.2  | 0.05 | 0.15 | 0.05 |
| Sporotermin      | 0.075| 0.075| -    | -    | -    | -    |

According to N.N. Ilyinsky, et al. [4], the presence of (a) micronuclei in cells of peripheral blood is natural, whereas the presence of other types is the result of cytogenetic disorder in fish organism. Besides, the sizes of micronuclei may indicate changes in the chromosome set. Thus, the presence of cells with large micronuclei is generally connected with spindle apparatus disorders, while the cells with small micronuclei are mainly caused by structural aberrations of chromosomes. The formation of cells with micronuclei of (b) and (e) types correlates with disorders in the structure of chromosomes. The
formation of cells with micronuclei of (d-I), (d-II) and (e) types is caused by the lag of chromosomes in meta- or anaphase.

4. Discussion
One of the factors ensuring the formation of micronuclei in erythrocytes of fish grown in industrial aquaculture are nitrites, which can accumulate in recirculating aquaculture systems as a result of wrong functioning of the second nitrification stage in water. When nitrites are accumulated in water fish suffer from methemoglobinemia since nitrites interact with hemoglobin of blood and the bivalent iron is oxidized [12-14]. This causes the formation of methaemoglobin, which is not able to transfer oxygen. This disturbs normal respiration of cells and body tissues, may lead to hypoxia, as a result lactic acid and cholesterol are accumulated, the intensity of protein synthesis decreases, including tubulin ensuring the formation of microtubules of spindle apparatus and chromosome disjunction [10, 15]. Besides, in the conditions of water pollution with nitrites and contamination of industrial aquaculture the presence of micronuclei in erythrocytes of fish can be caused by the delay of a cellular cycle at the stages of meta- and anaphases resulting from the inhibiting effect of nitrites on DNA reparation or blocking of tubulin synthesis. Therefore, the assessment of cytogenetic homeostasis can indicate the condition of an organism, its adaptive ability, as well as the level of cytopathological changes in cells due to various damaging factors.

Sporoterin decreases the disorders of cytogenetic homeostasis. Thus, the number of cells with micronuclei of (a) type decreases from 0.4% to 0.075%. There are no cells with micronuclei of (b), (c), (d-I), (d-II) and (e) types against the background of Sporoterin application as a feed additive.

5. Conclusions
The study of African sharptooth catfish via cytogenetic method showed that the probiotic Sporoterin reduces the frequency of micronuclei in erythrocytes of peripheral blood thus ensuring cytogenetic homeostasis of African sharptooth catfish (*Clarias gariepinus*).

6. Acknowledgements
The study was supported by the Russian Foundation for Basic Research, grant No. 18-016000127.

References
[1] Artemenkov D V2013 *Cultivation of sharptooth catfish (Clarias gariepinus) on compound feeds with probiotic additives subtilis in the conditions of recirculation system*: Dissertation, Moscow
[2] Kotova E A, Pyshmantseva N A, Osepchuk D V, Pyshmantseva A A and Tkhakushinova L N 2012 Probiotics in aquaculture. *Collection of scientific works of the All-Russian Research Institute of Sheep and Goat Breeding*, 3(1-1) 100–103
[3] Romanov V V, Romanova E M, Lyubomirova V N and Mukhitova M E 2018 Designing functional fish product in the conditions of industrial aquaculture. *USAA Bulletin* 1 151–156
[4] Ilyinskikh N N, Ksents A S, Ilyinskikh V N 2011 *Micronucleus test in the assessment of cytogenetic instability* (Tomsk. TSPU) pp. 312
[5] Kryukov V I and Kochkaryov P V 2013 Frequency of micronuclei in blood cells of fish living in fresh reservoirs of the Taimyr Peninsula. *Education, Science and Production* 1 35–37
[6] Alimba C G, Salju Joseph and Ubani-Rex O A 2015 Cytoxicity and histopathological assessment of Lekki Lagoon and Ogun River in Synodontis clarias (Linnaeus. 1758) *Toxicological & Environmental Chemistry* 97(2) 221–234
[7] Romanova E M, Spirina E V and Spirina T A 2011 Assessment of stability of development and cytogenetic homeostasis of Rana ridibunda Pall of Ulyanovsk region. *News of Samara Scientific Center of the Russian Academy of Sciences*. 13(1) 123–126
[8] Coico R, Sunshine G and Benjamin E 2003. *Immunology. A short Course* (Hoboken, NJ: Wiley-Liss Publication), pp. 237.
[9] King R W 2008 When 2+2=5: The origins and fates of aneuploid and tetraploid cells. *Biochimica et biophysica acta*. **1786**(1) 4-14

[10] Witzczak M, Kociszewska I, Wilczynski J 2010 Evaluation of chromosome aberrations. sister chromatid exchange and micronuclei in cultured cord-blood lymphocytes of newborns of women treated for epilepsy during pregnancy. *Mutation Research*. **701**(2) 111-117

[11] Harabawy Ahmed S A and Ibrahim Ahmed Th A 2014 Sublethal toxicity of carbofuran pesticide on the African catfish *Clarias gariepinus* (Burchell. 1822): Hematological, biochemical and cytogenetic response. *Ecotoxicology and Environmental Safety*. **103** 61–67

[12] Decordier I and Kirsch-Volders M 2006 The in vitro micronucleus test: from past to future. *Mutation Res*. **607**(1) 2–4

[13] Turchenyuk O V, Tomshina O L, Kalkov A P Micronucleus test to assess the ecological situation of the environment. *Omsk Scientific Bulletin*. **6**(42) 293–295

[14] Shakhtamirov I I, Kravtsov V Yu and Terletsky V P 2014 Micronucleus test at erythrocytes of fish living in zones of resistant organic pollutants in the river basin Terek. *News of St. Petersburg State Agricultural University* **34** 89–92

[15] Pashkov A N 2016 Micronucleus test: past, present and future. *Bulletin of Voronezh State University. Series: Chemistry. Biology. Pharmacy* **3** 150