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A. B. Bigaliev[1], B. O. Bekmanov[2], A. K. Jiyenbekov[1], B. E. Shimshikov[1], A. N. Kozhakhmetova[1]

[1]Kazakh National University n. al-Farabi, Almaty, Kazakhstan,
[2]RSE "Institute of General Genetics and Cytology"
Science Committee Ministry Education and Science Republic of Kazakhstan, Almaty, Kazakhstan.
E-mail: aitkhazha@gmail.com,zh-ai-bek@mail.ru, Batyrgeldi.Shimshikov@kaznu.kz, ayzada.1983@mail.ru

MOLECULAR-GENETICAL INVESTIGATION OF OIL POLLUTION EFFECT ON AGUABIOTA OF KAZAKHSTANY ZONE OF CASPEAN SEA

Abstract. The Study of the impact of pollutants on the aquatic ecosystem for the purpose of biological indication of areas subject to anthropogenic pollution is extremely important for the Caspian sea. The article presents the restriction DNA analysis of fishes (*Neogobiusgorlap*) and polychaetes (*Nereisdiversicolor*) from three habitats of the coastal zone of the Caspian sea. Currently, the method of DNA analysis, polymerase chain reaction (PCR), developed in 1985, is widely used to assess genome stability [1]. The sensitivity of PCR makes it possible to successfully analyze even degraded DNA from Museum and archaeological samples. In this paper, we used the RAPD-PCR analysis method. In the total selected sample of 3 biotopes, 4 to 11 fragments were evaluated. Visual analysis of DNA fragments obtained for samples had a very high degree of differences (difference). All selected primers showed a different picture of inter-species differentiation. It is absolutely reliable markers (interbreeding specific DNA fragments) to identify phenotypic interbreeding, studied fishes. The most important primers for the samples were OPA-09 and OPA-10. During the analysis of polymerase chain reaction spectra with polymorphic and monomorphic DNA of fish caught in sites with different levels of pollution were found. The specific properties of the DNA spectra of the studied fish and polychaetes were determined. A unique DNA fragment was found in polychaetes from the polluted environment, which can be used as a DNA marker.

Key words: RAPD-PCR, DNA, electrophoresis, enzyme, restrictase, primers, polymerase chain reaction, polymorphism.

Introduction. Restriction DNA analysis of any organism is widely used in molecular genetic research and is one of the most important tools in the study of violations in the DNA molecule. DNA molecule cleavage products are analyzed using gel electrophoresis in agarose or polyacrylamide and the resulting pattern of DNA molecule fragment separation in the form of a specific, different for different enzymes, a set of bands and is the result of restriction analysis of the studied DNA. Many restrictase allow cleavage of DNA at more than 150 sites of recognition. Restriction analysis is carried out for a variety of DNA, ranging from small fragments of several tens of nucleotide pairs, and up to the entire eukaryotic genomes of more than 1 billion base pairs. It was found that in most cases the cleavage of chromosomal DNA by restriction endonucleases resulted in the formation of clearly visible fragments of a certain length, which allowed to judge about genome disorders of the studied animals, in particular polymorphism in individuals, which living at strong polluted area [1-3].

Actuality of the work. The study of the impact of pollutants on the ecosystem for the purpose of bioindication of technogenic contaminated areas is of extreme relevance for the Caspian sea area. Molecular genetic analysis of species of fish and the polychaetes from the coastal zone of the Caspian sea for assessing the impact of pollutants on the stability of the genome for the first time. Determination of the accumulation of polycyclic aromatic hydrocarbons (PAHs) and their metabolites as specific xenobiotics in

the area of oil production, processing and transportation is an extremely urgent problem of the Kazakhstan shelf of the Caspian sea.

Purpose of research. The purpose of this work is to conduct a reconnaissance study of the effect of oil pollution on the genome stability of test objects by molecular genetic analysis methods

Tasks:

- analysis by polymerase chain reaction of DNA spectra of fish and polychaetes selected from the coastal zone of the Caspian sea with different levels of pollution;
- quantitative and qualitative assessment of the isolated DNA from the body of test objects

Research materials and methods. The objects of research were selected goby fish (Neogobius-gorlap) and polychaetes (Nereisdiversicolor) caught in the coastal zone of the Caspian sea. Three points were selected for the analysis by polymerase chain reaction of fish and polychaete DNA spectra: Atyrau, of the river Ural delta and the coastal zone of the Caspian sea with different levels of pollution. These types of aquatic organisms fully meet the requirements for biomonitoring objects: widespread distribution in the reservoir, well-studied biology, do not make long migrations. The material for the study served as the fins of fish and the tissue of the polychaetes.

Quantitative and qualitative assessment of the isolated DNA was performed using spectrophotometric and electrophoretic analysis. For spectrophotometric analysis, adsorption of aqueous DNA solutions was measured at three wavelengths: 260 nm, 280 nm and 320 nm. The size of DNA molecules and the presence of RNA impurities were determined by electrophoresis in 0.7% agarose gel after staining with bromide ethidium. Visualization of DNA, RNA was carried out using a transilluminator in ultraviolet light.

Treatment of DNA by enzymes. Ferments produced of company “Fermentas”, Vilnius, Lithuania were used in the work." The cleavage of DNA was carried out in the manufacturer's recommended buffers for restriction of DNA at the optimum temperature for 16 hours. Restriction was carried out in 20 μl of the reaction mixture to which 5 μl of DNA and 0.5 μl of the enzyme were added. Activity of the enzymes used: AluI - 10 u / μl, EcoRI - 10 u/μl, BsuRI - 10 u / μl.

Electrophoresis. To identify fragments of length from 40 to 2000 p.b. used the electrophoresis in 8% polyacrylamide gel (on the track we applied 5 μl of the treated DNA) ("Sigma", USA). After the electrophoresis, DNA visualized with ethidium bromide and photographed under UV light.

DNA sample. For genomic DNA extraction a set of reagents QIA amp DNA Mini Kit (Qiagen, USA) was used. Quantitative and qualitative assessment of the isolated DNA was performed using DNA photometer (Biofotometer Plus, Eppendorf, Germany) and electrophoretic analysis. For photometric analysis, adsorption of aqueous DNA solutions was measured at three wavelengths: 260 nm, 280 nm and 320 nm. The size of DNA molecules, as well as the presence of RNA impurities, was determined by electrophoresis in 0.7% agarose gel after staining with bromide ethidium. Visualization of DNA, RNA was carried out using a transilluminator in ultraviolet light. PCR mixture with Taq polymerase, PCR Master Mix (Thermo Scientific, Lithuania) was used for DNA amplification of the studied and control samples. Amplification was performed automatically on the programmable master cycler nexus Gradient amplifier (Eppendorf, Germany) using the "hot start"method. The tubes with reagents were placed in an amplifier heated to a temperature of 93-94°C. This technique allows to avoid non-specific annealing of primers.

RAPD-PCR analysis. Polymerase chain reaction was carried out with ten members oligonucleotide primers synthesized in RSE "Institute of General Genetics and Cytology" (Kazakhstan) on a synthesizer ASM-800 of Bioset company, (Russia) (table). To minimize the error, the reaction was optimized by selecting the necessary concentrations of each component and preparing the total mixture for the entire sample.

The PCR reaction was carried out in the following temperature mode: initial denaturation at 94°C for 2 min, 40 cycles consisting of four stages, including 45 C at 92°C, 30 C at 37°C, 15 C at 45°C and 2 min at 72°C. the Reaction completed a 10-minute elongation stage at 72°C. Negative reaction control (contamination test) contained a reaction mixture without the addition of DNA.

Electrophoresis. Electrophoresis of amplified DNA fragments was carried out in 2% agarose gel in Tae_buffer (0.89 M Tris, 0.1 M sodium acetate, 0.05 M EDTA), pH 7.8 with bromide etide (5 μg/ml) and was photographed in transmitted UV light. The size of each fragment was determined by comparison with marker DNA fragments Gene Ruller 100 kbDNALadder (ThermoScientific, Lithuania).

Primers used for RAPD-PCR DNA analysis of fish and polychaetes

Primer	5′ → 3′
OPA-02	TGCCGAGCTG
OPA-09	GGGTAACGCC
OPA-10	GTGATCGCAG
OPA-11	CAATCGCCGT

Results. At the first stage of the research, the most informative primers for PCR analysis based on literature data were selected. Primers with the nucleotide sequence OPA-02 (TGCCGAGCTG); OPA-09 (GGGTAACGCC); OPA-10 (GTGATCGCAG) and OPA-11 (CAATCGCCGT). Then the variability of randomly amplified DNA was analyzed by RAPD-PCR method with selected standard 10-nucleotide primers.

During the analysis of the DNA spectra of fish obtained by polymerase chain reaction, caught in the vicinity with different levels of pollution, both polymorphic and monomorphic DNA were found. According to the literature, such monomorphic DNA should be distinguished from polymorphic and considered as a manifestation of genetic monomorphism at the DNA level [4, 5]. The features of the DNA spectra of the studied fish and polychaetes in two areas with different levels of pollution of the environment (biotopes) were revealed. A unique DNA fragment was found in individuals living in a more polluted environment. The results of studies of the influence of anthropogenic factors on the features of the DNA spectra of the fish studied, obtained by PCR analysis are shown in figure 1.

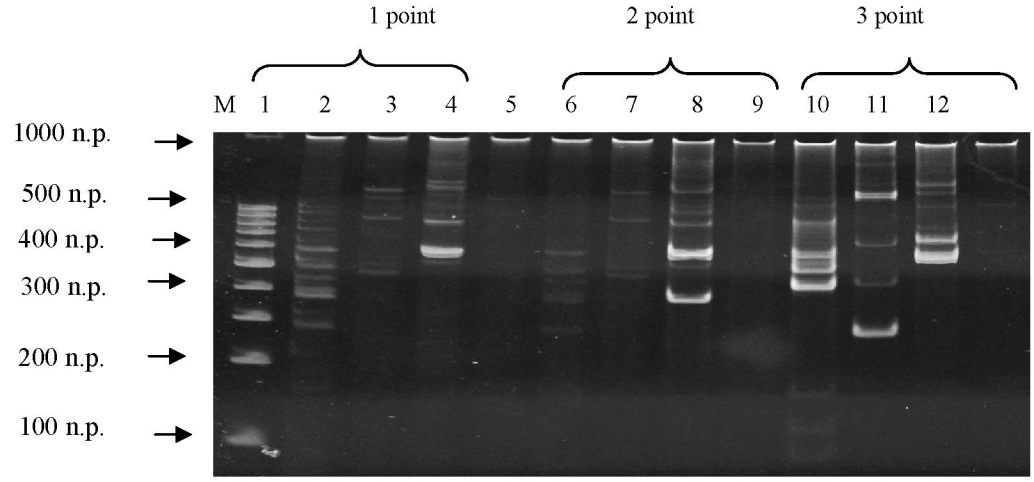

Figure 1 – RAPD - polymorphism of fish detected with primers OPA-02, OPA-09, OPA-10 and OPA-11.
1 point – the coastal area of the Caspian sea; 2 – point Delta of the Ural river; 3 point – coastal area of the city of Atyrau;
M – DeMarker (GeneRuller 100 kb DNA Ladder). 1 – the primer OPA-02; 2 – primer OPA-09; 3 – primer OPA-10;
4 – primer OPA-11; 5 – primer OPA-02; 6 – primer OPA-09; 7 – primer OPA-10; 8 – primer OPA-11;
9 – primer OPA-02; 10 – primer OPA-09; 11– primer OPA-10; 12 – primer OPA-11

The total sample of 3 selected points revealed from 4 to 11 fragments. In visual analysis of DNA fragments obtained for species samples had a very high degree of dissimilarity (differences). All selected primers showed different patterns of inter-species differentiation. But it is absolutely reliable markers (i.e. hybridizing DNA fragments) to identify phenotypic hybrids studied fish have been identified. For the studied samples, the most indicative primers were OPA-09 and OPA-10. Figure 1 shows distinct strips (DNA fragments) of different sizes.

Thus, during the analysis of the DNA spectra of fish obtained by polymerase chain reaction, caught from different degrees of contamination of biotopes, polymorphic DNA was found. In the studied fish samples monomorphic DNA was also revealed. Figure 1 shows that polymorphic DNA markers may be responsible for different characteristics of individuals.

The next stage of the study was the analysis of polychaetes caught in the same biotopes as fish. The same primers OPA-02, OPA-09, OPA-10 and OPA-11 were used. The studies have shown that the DNA spectrum of polychaetes contains from 6 to 9 randomly amplified DNA fragments length from 100 to 1200 nucleotide pairs (figure 2). It should be noted that the fragment on the 12 sample, which contains more than 1000 BP, is the most indicative and is of interest. DNA fragments found in other individuals can serve as markers of different processes and characteristics that distinguish these individuals.

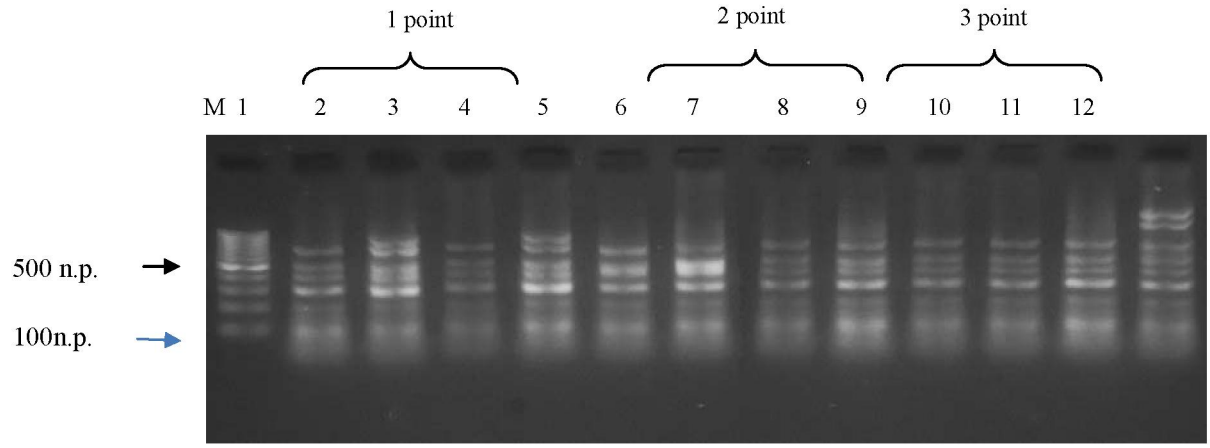

Figure 2 – RAPD-polymorphism of policeto identified by using primers OPA-02, OPA-09, OPA-10 and OPA-11.
1 point – the Delta of the Ural river; 2 point – intensely polluted coastal area of the Caspian sea;
3 point – low polluted coastal area of the Caspian sea; M – DeMarker (GeneRuller 100 kb DNA Ladder).
1 – the primer OPA-02; 2 – primer OPA-09; 3 – primer OPA-10; 4 – primer OPA-11; 5 – primer OPA-02; 6 – primer OPA-09;
7 – primer OPA-10; 8 – primer OPA-11; 9 – primer OPA-02; 10 – primer OPA-09; 11– primer OPA-10; 12 – primer OPA-11

During the analysis of the DNA spectra of fish obtained by polymerase chain reaction, caught from different degrees of contamination of biotopes, polymorphic DNA was found. Monomorphic DNA was also revealed in the studied fish samples. DNA spectrum of polychaetes contains from 6 to 9 randomly amplified DNA fragments with length from 100 to 1200 nucleotide pairs. Fragment 12 the sample contains more than 1000 BP is the most striking manifestation of polymorphism. In turn, DNA fragments detected in other individuals can serve as markers of different processes and characteristics that distinguish these individuals. A DNA fragment 300 BP long was detected, which was found in all polychaetes (100% frequency of occurrence). This fragment can be considered as a manifestation of genetic monomorphism at the DNA level. Later it can also be used as a molecular marker for specific species under study.

Discussion. From the literature data it follows that RAPD-markers are localized mainly in the non-coding region of DNA, because it it is the vast majority of the eukaryotic genome. The rate of mutation in non-coding DNA is about twice higher than in coding. In addition, RAPD markers are sometimes amplified from regions of repetitive DNA and thus may reflect high rates of their mutation, which can be induced by radiation or chemical factors of environmental pollution. On this basis, a method of Express identification of species belonging of living sturgeon tissue samples, products of their processing, including caviar, using a multiplex polymerase chain reaction (IGCC), performed in one tube, which allows simultaneously amplifying the unique sites of the mitochondrial genome of different species of sturgeon to obtain species-specific multi-dimensional amplicons, divided in electrophoresis into polyacrylamide or agarose gel [6,7].Thus, in the works of De Salle, 1996, Birstein, 1998, unlike the previously proposed test systems, the developed technique allows to discriminate unambiguously against Siberian and Russian sturgeons, including carriers of the mitochondrial "baerii-like" haplotype, which was previously impossible, and repeatedly led to an erroneous interpretation of the origin of caviar products exported from the Caspian region to the United States and other countries [8, 9]. Various methods of DNA polymorphism assessment depending on the goals are successfully used at different levels: from the individual and the population to the orders and the superorder categories. In many cases, variable DNA markers can detect genetic differences that are not recognized by protein electrophoresis.

RAPD is a DNA polymorphism. The RAPD (Random Amplified Polymorphic DNA) method is based on PCR-amplification of anonymous DNA sites using one, usually short (up to 10 nucleotide pairs, n) primer of arbitrary sequence, which induces in each reaction the synthesis of up to several tens of DNA fragments of random localization. The ability to investigate the polymorphism of the entire genome without prior knowledge of specific DNA sequences is a major advantage of RAPD analysis. The high level of polymorphism determined by the RAPD-method is quite informative in population studies, to identify hidden genetic polymorphism in lines and closely related species, as well as individual identification [9]. It should be noted that RAPD-markers are inherited as dominant features, which does not allow to distinguish the dominant homozygote from heterozygote and recessive homozygote from unamplified sequence. To overcome this disadvantage of the method, an original mathematical apparatus was developed, with which the frequency of the recessive conditional "zero-allele"is taken into account. The low reproducibility of RAPD-analysis due to non-specificity deserves attention from the negative sides primers inducing amplification of bands of different intensity, formation of heteroduplexes. The application of this technique is limited by the high quality requirements of the matrix and the potential non-homologation of RAPD bands (one fragment may contain different nucleotide sequences of the same size on an electrophoregram). Note that the development of the method emphasized the potential possibility of constructing with the help of RAPD-markers maps of adhesion to specific loci. However, the progress in this area is more than modest, besides such work requires quite expensive large-scale screening. For example, 310 random primers of different structures were used to identify DNA markers that diagnose the gender of Beluga, but none of the 4146 obtained bands was linked to the sex [10, 11]. RAPD-PCR is a PCR with random amplification polymorphic DNA is used to study the variability of similar genetic sequence of organisms, for example, different varieties of crops, breeds of dogs or closely-related microorganisms. This method usually uses one primer of small size (about 10 BP). This primer may be partially complementary to random DNA regions of the organisms under study. The literature data suggest that the increased content of PAH, heavy metals (copper, iron and manganese, etc.) may cause an increase in the level of DNA damage in fish erythrocytes [11-13]. Among environmental pollutants, PAHs and metals are of particular concern due to their potential toxic effects and bioaccumulative potential in aquatic ecosystems. Thus, the genotoxicity of copper and zinc is shown in a separate and combined action using a micro-nuclear test, copper causes an increase in the level of micronuclei in fish Oncorhynchusmykiss. The increase in the level of DNA damage in erythrocytes of fish Prochiloduslineatus by the action of aluminum is shown by the method of DNA-comets. A possible mechanism of iron toxicity may be the induction of DNA damage due to the generation of free oxygen radicals, which can cause site-specific oxidative damage [14, 15].

Conclusion. Thus, hydrobionts (fish and polychaetes) can be the most adequate object for the assessment of water pollutants, as they metabolize and accumulate in the body the chemical compounds contained in water. In our case, both fish and polychaetes react to toxic compounds similar to higher vertebrates. They can be used for screening chemical compounds, potentially mutagenic and carcinogenic to humans.

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REFERENCES

[1] Shibata D.K. Polimeraznaja cepnaja reakcija i molekuljarno-geneticheskij analiz biotopov. Molekuljarno-klinicheskaja diagnostika. Metody. M.: Mir, 1999. P. 395-425.

[2] Konovalova A.N. i dr. Polimeraznaja cepnaja reakcija (principy, vozmozhnosti) // Izvestija NAN RK. 2011. N 5. P. 60-71.

[3] Venter J. et al. The sequence of the human genome // Scince. 2001. Vol. 291. P. 1304-1311. DOI: 10.1126/science.1058040.

[4] Zelenina D.A., Hrustaleva A.M., Volkov A.A. Sravnitel'noe issledovanie populjacionnoj struktury i opredelenie populjacionnoj prinadlezhnosti nerki (Oncorhynchusnerka) Zapadnoj Kamchatki s pomoshh'ju RAPD-PCR i analiza polimorfizma mikrosatellitnyh lokusov // Genetika. 2006. Vol. 42. P. 693-704.

[5] Bigaliev A.B., Kobegenova S.S., Vasil'ev V., Vasil'eva E., Imentai A., Shametov A. Study of Caspian Goby Neogobius sp. Karyotipe Flexibility from Several Biotops // Journal of Life Sciences. USA. May 2014. Vol. 8. P. 442-446. ISSN 1934-7391.

[6] Bigaliev A.B., Ishanova N., Bigaliev A.A., Biyasheva Z.B. Ecological assessment of oil-gas producing area in Kazakhstan zone of Caspian sea and using bioremediation technology for cleaning of high level oil polluted sites. Kyoto, 2008, Abstracts.

[7] Rozhkovan K.V., Chelomina G.N., Rachek E.I., Molekuljarnaja identifikacija i osobennosti geneticheskogo raznoobrazija mezhvidovyh gibridov amurskogo osetra (Acipenser schrenckii × A. baerii, A. baerii × A. schrenckii, A. schrenckii × A. Ruthenusi A. ruthenus × A. schreckii) po dannym izmechivosti mul'tilokusnyh RAPD-markerov // Genetika. 2008. Vol. 44. P. 1453-1460.

[8] DeSalle R., Birstein V.J. PCR identification of black caviar // Nature. 1996. N 377. P. 673-674.

[9] Birstein V.J., DeSalle R. Molecular phylogeny of Asipenser // Molecular Phylogenetics and Evolution. 1998. N 8. P. 141-155.

[10] Bigalyev A.B., Shalabaeva K.Z., Kulumbetov A.K., Jiyenbekov A.K., Adylova L.M., Kozhahmetova A.N., Myrzakhan A.G., Ishanova N.E. Ecology-genetical evaluation of radiation and pollution (Associated with heavy metalls) on biota and man. Review // International Journal of research advanced. Impact Factor: 6.118. October 2017. Vol. 5, Issue 10. P. 819-825. DOI: 10.21474/IJAR01/5591.

[11] Timoshkina N.N., Rynza E.T., Usatov A.V. DNK–identifikacija «baerii-like» mitotipa v azovskoj populjacii russkogo osetra (Acipensergueldenstaedtii) // Sb. nauch. tr. S#ezda Ukrainskogo obshhestva genetikov i selekcionerov. Kiev: Logos, 2007. Vol. 1. P. 326-329.

[12] Altuhov Ju.P., Salmenkova E.A. Polimorfizm DNK v populjacionnoj genetike // Genetika. 2002. Vol. 38, N 9. P. 1173-1195.

[13] Keyvanshokooh S., Pourkazemi M., Katbassi M.R. The RAPD technique failed to identify sex-specific sequences in beluga (Husohuso) // J. Appl. Icht. 2007. Vol. 23. P. 1-2. DOI: 10.1111/j.1439-0426.2006.00798.x

[14] Abdullaeva N.M., Gabibov M.M. Sostojanie membran jeritrocitov perifericheskoj krovi ryb pri vozdejstvii tjazhelyh metallov i syroj nefti // Izvestija vysshih uchebnyh zavedenij. Severo-Kavkazskij region. Serija: Estestvennye nauki. 2011. N 5. P. 50-54.

[15] Bagrjanceva Ja.V. Jekotoksikologicheskaja ocenka pribrezhnyh morskih akvatorij na osnove metoda «DNK-komet» // Sovremennye naukoemkie tehnologii. 2005. N 5. P. 92-93.

А. Б. Бигалиев[1], Б. О. Бекманов[2], А. К. Джиенбеков[1], Б. Е. Шимшиков[1], А. Н. Қожахметова[1]

[1]Әл-Фараби атындағы Қазақ ұлттық университеті, Алматы, Қазақстан,
[2]РММ «Жалпы генетика және цитология институты» Қазақстан Республикасы Білім және ғылым Министрлігінің Ғылым Комиеті, Алматы, Қазақстан

КАСПИЙДІҢ ҚАЗАҚСТАНДЫҚ АУМАҒЫНЫҢ АКВАБИОТАСЫНА МҰНАЙМЕН ЛАСТАНУ ӘСЕРІН МОЛЕКУЛАЛЫҚ-ГЕНЕТИКАЛЫҚ ЗЕРТТЕУ

Аннотация. Антропогендік ластануға ұшыраған аймақты биологиялық индикациялау мақсатында ластаушы заттардың су экожүйесіне әсерін зерттеу Каспий теңізі үшін өте маңызды. Мақалада Каспий теңізінің жағалау аймағының үш ареалынан алынған балық (*Neogobiusgorlap*) пен полихеттің (*Nereis diversicolor*) ДНҚ-ның рестрикциялық талдауы келтірілген. Қазіргі таңда геном тұрақтылығын бағалау үшін 1985 жылы жасалған ДНҚ талдауының полимеразды тізбекті реакция (ПТР) әдісі қолданылады [1]. ПТР сезімталдығы мұражайлар мен археологиялық ескірген ДНҚ-ды да талдауға мүмкіндік береді. Бұл жұмыста RAPD-PCR талдау әдісін қолдандық. Үш биотоптан алынып іріктелген 4-тен 11-ге дейін фрагменттер бағаланды. Үлгілерден алынған ДНҚ фрагменттерін визуалды талдау кезінде үлкен айырмашылықтар байқалды. Алынған барлық праймерлер тұраралық дифференциацияның әртүрлі көріністерін көрсетті. Бұл зерттелген балықтардың фенотиптік гибридтерін біртектендіру үшін сенімді маркерлер. Зерттелген үлгілер үшін OPA-09 және OPA-10 біршама маңызды праймерлер болып табылды. Полимеразды тізбекті реакция талдауы кезінде ластану деңгейі әртүрлі жерлерден алынған балық ДНҚ-да полиморфты және мономорфты спектрлер байқалды. Зерттелген балық пен полихеттердің ДНҚ-спектрлерінде арнайы қасиеттері анықталды. Ластанған аймақтан алынған полихеттерде ерекше ДНҚ-фрагмент табылды, ол фрагментті ДНК маркер ретінде қолдануға болады.

Түйін сөздер: RAPD-PCR, ДНҚ, электрофорез, фермент, рестриктазалар, праймерлер, полимеразалы тізбекті реакция, полиморфизм.

А. Б. Бигалиев[1], Б. О. Бекманов[2], А. К. Джиенбеков[1], Б. Е. Шимшиков[1], А. Н. Кожахметова[1]

[1]Казахский национальный университет им. аль-Фараби, Алматы, Казахстан,
[2]РГП «Институт общей генетики и цитологии» КН МОН РК, Алматы, Казахстан

МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКОЕ ИССЛЕДОВАНИЕ ВЛИЯНИЯ НЕФТЯНОГО ЗАГРЯЗНЕНИЯ НА АКВАБИОТУ КАЗАХСТАНСКОЙ ЗОНЫ КАСПИЯ

Аннотация. Изучение влияния загрязняющих веществ на водную экосистему с целью биологической индикации территорий, подверженных антропогенному загрязнению крайне актуально для Каспийского моря. В статье представлен рестрикционный анализ ДНК рыб (*Neogobiusgorlap*) и полихет (*Nereisdiversicolor*) из трех ареалов прибрежной зоны Каспийского моря. В настоящее время для оценки стабильности генома широко используется метод анализа ДНК, полимеразной цепной реакции (ПЦР), разработанный в 1985 году [1]. Чувствительность ПЦР позволяет успешно анализировать даже деградированную ДНК из музейных и археологических образцов. В данной работе мы использовали метод анализа RAPD-PCR. В общей отобранной выборке из 3-х биотопов оценивали от 4 до 11 фрагментов. Визуальный анализ фрагментов ДНК, полученных для образцов, имел очень высокую степень различий (различие). Все выбранные праймеры показали различную картину межвидовой дифференциации. Это абсолютно достоверные маркеры (gibridspecies DNA fragments) для идентификации фенотипических гибридов, изученных рыб. Для исследуемых образцов были наиболее важными праймеры OPA-09 и OPA-10. Во время анализа полимеразной цепной реакции были обнаружены спектры с полиморфной и мономорфной ДНК рыб, пойманных в местах с различным уровнем загрязнения. Определены специфические свойства ДНК-спектров исследуемых рыб и полихет. Обнаружен уникальный ДНК-фрагмента у полихет из загрязненной среды обитания, который может быть использован в качестве ДНК маркера.

Ключевые слова: RAPD-PCR, ДНК, электрофорез, фермент, рестриктазы, праймеры, полимеразная цепная реакция, полиморфизм.

Information about authors:

Bigaliev A.B. – professor of Kazakh National University named after al-Farabi, Almaty, Kazakhstan; aitkhazha@gmail.com

Bekmanov B.O. – researcher of the RSE of the Institute of General Genetics and Cytology of the Committee of Science Ministry of Education and Science of the Republic of Kazakhstan, Almaty, Kazakhstan; zh-ai-bek@mail.ru

Jiyenbekov A. K. – doctoral student of Kazakh National University named after al-Farabi, Almaty, Kazakhstan

Shimshikov B. E. – associate professor of the Kazakh National University named after al-Farabi, Almaty, Kazakhstan; Batyrgeldi.Shimshikov@kaznu.kz

Kozhakhmetova A.N. – researcher of the Kazakh National University named after al-Farabi, Almaty, Kazakhstan; ayzada.1983@mail.ru