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The Modeling of Aquatic Organisms Identification by Marker Proteins.

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ABSTRACT

One of the most relevant methods of biological indication of ecological environment is the characteristics of quantitative and qualitative composition of indicator organisms. On basis of method of biological indication is estimated the ecological condition of environment, including water bodies by studying of species-indicators inhabiting the researched medium, determined up to present time visually via microscope with extraction of each organism from water sample. As alternative we proposed to apply the method of identification and characteristic of aggregation of aquatic organisms in water sample by marker proteins. In this work is conducted the modeling of saprobity estimation of water bodies of three lakes (Upper, Middle and Lower Kaban, Kazan, Russia) via semi-quantitative identification of aquatic organisms by marker proteins on example of indicator organisms. Modeling is conducted on basis of estimation of content in model samples of bacterial proteins, conditionally accepted as according to marker proteins of aquatic organisms. Estimation of proteins content in sample is conducted in semi-quantitative manner on basis of method of immonoblotting and further analysis of intensity of protein stripes fluorescence for calculation of relative quantitative composition in the sample. By results of research the anticipated result of identification model of aquatic organisms by proteins completely matches experimental data and accords to estimation of experts on ecological condition of Kaban water bodies by method of biological indication.

Keywords: marker proteins, model of aquatic organisms identification by marker proteins, indicator aquatic organisms

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INTRODUCTION

It is known that one of highly informative methods of estimation of ecological environment is an application of biological indication method, i.e. detection of non-specific reaction of living organisms for change of condition of their life environment, for example - pollution or contamination [1, 2].

On basis of method of biological indication is estimated the ecological condition of environment, including water bodies by studying of species-indicators inhabiting the researched medium, determined up to present time visually via microscope. Identification of organisms by morphological signs depends on qualification and subjectiveness of expert, accompanied by large labor cost and significant time cost [4]. Besides, the method of biological indication on basis of morphological signs has an insufficient resolution ability due to existence of species-doubles having a similar external structure [5] and existing in nature if heteromorphic life cycle of some living organisms, i.e. occurring within life change of external appearance of organism defined [6].

Subjectivity of results of determination of indicator species of fresh-water organisms in water sample reduces the accuracy of estimation of ecological condition of water bodies, accompanied by unintentional mistakes, which frequently lead to need in repeated biological indication with additional costs of time, with involvement of high-qualified specialists. As result the acceptance of correct decision for conduction of sanitary measures is delayed.

As an alternative to method of biological indication by morphological signs with extraction of each organism from water sample we proposed to use the method of biological indication of aquatic organisms by marker genes on basis of contemporary methods of bio-informatics and molecular genetics, such as DNA bar-coding [7, 8].

In this work are shown results if research on identification of aquatic organisms by marker proteins basing on method of enzyme-linked immunosorbent assay, ELISA, which is used for determination of presence of anti-genes of diverse infections germs in plants, animals, man and other materials by detection of unique antigenes to marker proteins inherent to these germs[9] and methods of bioinformatics on work with proteins [10]. The model is proposed for identification of aggregation of aquatic organisms in water sample by marker proteins and bu this is significantly different from identification of aquatic organisms by market genes that is conducted for each organism separately in water sample.

Therefore, the objective of the work s the increasing of precision and accuracy of results of estimation of ecological condition of water environment with application of marker proteins, replacement of materials of local analytical researches with subjective results with results of objective instrumental researches, increase of labor productivity, shortening of terms of estimation of pollution level and diagnostic of condition of water environment, increase of effectiveness of nature protection activity.

MATERIALS AND METHODS

For identification of organisms by proteins as model objects in the work were used recombinant proteins TnrA, GlnK and glutamine of sythesis (GS) from recombinant strains *Escherichia coli* BL21with plasmides: pET15b-TnrA, pDG148-GlnK and pGP177 [11]. Strains of *E.coli* were cultivated with swinginf at temperature of 37ºC in medium LB (triptone – 1.0%; yeastrel – 0.5%; NaCl – 0.5%; pH 8.5 [12]). At growing of recombinant strains of *E.coli* with plasmides pET15b-TnrA, pDG148-GlnK and pGP177 ampicilline up to final concentration of 100 mkg/ml in medium was introduced. For induction of hyper-expression of protein to culture of cells being in exponential phase of growth ($ОП_{600}$=0.8) was added IPTG up to final concentration of 1 mM.

Sampleswith model proteins were divided by means of electrophoretic method of proteins division in 15% polyacrilamide gel by method of Laemmly [13]. Content of proteins TnrA, GlnK and GS in samples was estimated by semi-quantitative immunoblotting with use of antibodies gainst these proteins [11].

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RESULTS AND DISCUSSIONS

The modeling of aquatic organisms identification by marker proteins

Principle of work of model of aquatic organisms identification by marker proteins in comprised the fact that from the estimated water environment the organisms samples are collected, identification of species composition of organisms from sample is conducted with application of marker proteins via determination of qualitative and quantitative composition of marker proteins of indicator organisms in sample and the level of environment pollution is estimated in accordance of species composition of indicator organisms and their proportion in a sample with application of marker proteins. As indicator organisms for estimation of environment with application of marker proteins are used living organisms – zooplanktoonic organisms, fishes, zoobenthos, protists living in water environment [14].

Realization of mode is shown on example f research of zooplanktonic organisms of three lakes Kaban of Kazan of he Republic of Tatarstan. By information of ecologists, the lake Lower Kaban os characterized by more pollution (polysaprobic) than lake Middle Kaban (betamesosaprobic) and lake Upper Kaban (oligosaprobic) [15]. Therefore, lakes Kaban are representing the system of water objects with different estimation of ecological condition of water bodies and due to this are selected as model water bodies.

Previously by methods of bioinformatics in our work is shown that proteins CO1 of zooplanktonic and zoobenthos organisms, and proteins rbcL of phytoplanktonic organisms can be used as marker for identification of organisms of different zones of saprobity of water bodies from "very pure" to "very polluted" [16, 17].
We were basing on suggestion that at application of antibodies for market protein CO1 of three indicator organisms (*Daphnia pulex* – polysaprobic, *Brachionus calyciflorus* – betamesosaprobic and *Kellicottia longispina* – oligosaprobic) the results of analysis would look the following way (Fig. 1).

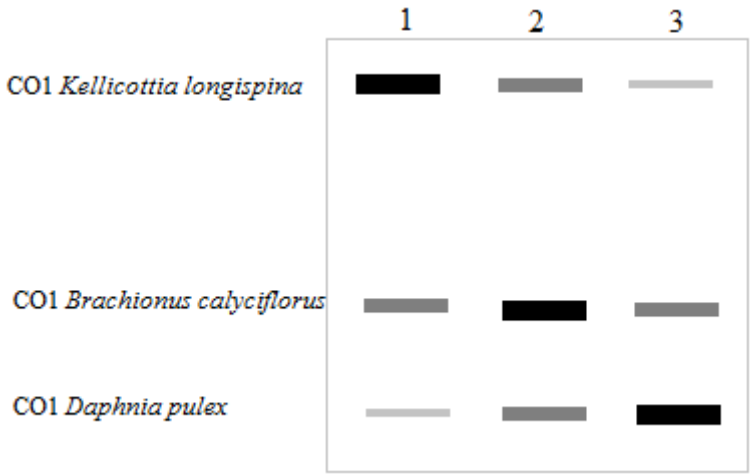

Figure 1 – anticipated results of aquatic organisms identification by proteins

In figure 1 is shown that the higher is the concentration of indicator organism's protein, the bigger is the quantity and biomass of aquatic organism, and the stronger is expressed the saprobity of the water body. For example, in tube No. 1 for lake Upper Kaban we will receive higher concentration of oligisaprobic organism, such as, for example, *Kellicottia longispina.* In tube No. 2 for lake Middle Kaban we will receive higher concentration of betamesosaprobic organism, such as, for example, *Brachionus calyciflorus.* And, finally, in tube No. 3 for lake Lower Kaban we received higher concentration of polysaprobic organism, such as, for example, *Daphnia pulex.*

Therefore, the more antibodies are used for analysis of indicative species of aquatic organisms, the more precise is probability of receiving of highly accurate result.

Verification of model of aquatic organisms identification by marker proteins

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Received results of computer analysis of possibility of aquatic organisms identification by marker proteins usually need the experuimental checking. but receiving of antibodies against proteins CO1 of 23 species of zooplankton, 19 species of zoobethos and proteins rbcL of 21 species of phytoplankton requires substantial financial costs that are rational only after receiving of satisfactory results on experimental model object. For imitation of aquatic organisms by marker proteins were selected 3 proteins from bacteria *Bacillus subtilis* (TnrA, GlnK and GS), which hyperproduction in cells of *E.coli* and antibodies were received earlier [11].
For verification of model of aquatic organisms identification by proteins we compared proteins CO1 of organisms from collected samples of lakes Kaban of Kazan to model proteins TnrA, GlnK and GS, received in course of experiment: CO1 *Brachionus calyciflorus* (betamezosaprobic) – TnrA, CO1 *Daphnia pulex* (polysaprobic) – GlnK и CO1 *Kellicottia longispina* (oligosaprobic) – GS, as anibodies were usedready antibodies *αTnrA, αGlnK* and *αGS* against proteins TnrA, GlnK and GS, conducted analysis of proteins concentration in sample by method of immunoblotting and gave the relative semiquantitative estimation of species in the sample.

For modeling of proportion on indicator organisms in water bodies of different saprobity, cells of *E.coli*, containing proteins GS, TnrA, and GlnK, were mixed in different proportions, according to quantitative proportion of respective indicator organisms (Table 1).

Table 1 – Proportional relations of proteins GS, TnrA, and GlnK at electrophoresis

	Well 1	Well 2	Well 3
GS	3	2	1
TnrA	2	3	2
GlnK	1	2	3

So, to first well corresponds the sample from lake Upper Kaban, second well - sample from lake Middle Kaban, and third well - sample from lake Lower Kaban. Than samples were divided in 15% polyamide gel and content of protein was analyzed by immunoblotting (Figure 2).

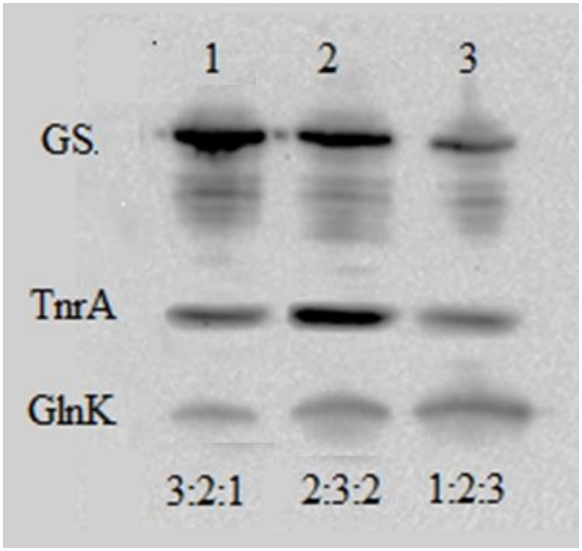

Figure 2 - Results of immunoblotting

As can be seen from Figure 2 , in first well, modeling the sample fro lake Upper Kaban, upper stripe is brighter, i.e. reflects the larger quantity of protein GS, which is compared to protein CO1 of *Kellicottia longispina* (oligosaprobic). In the second well, modeling the sample from lake Middle Kaban, the middle stripe is brighter, i.e. reflects the larger quantity of protein TnrA, which is compared to protein CO1 of *Brachionus calyciflorus* (betamesosaprobic). In the third well, modeling the sample from lake Lower Kaban, the lower stripe is brighter, i.e. reflects the larger quantity of protein GlnK, which is compared to protein CO1 of *Daphnia pulex* (polysaprobic).

By received analysis was conducted the estimation of proteins content in samples by intensity of fluorescence (Table 2). Analysis shown that the content of proteins was approximately according to initial content of proteins in samples (Table 1).

Table 2 – Proportional relations of proteins GS, TnrA, and GlnK at electrophoresis

	Well 1	Well 2	Well 3
GS:TnrA:GlnK	2.6:2.1:1.0	2.1:3.7:2.1	1.1:1.9:3.2

Therefore, the received experimental results accord to anticipated results represented in model of aquatic organisms identification by marker proteins (see Figures 1, 2). By summary of experiment the ecological condition of lakes Kaban can be estimated as: Upper Kaban - olihgosaptobicm Middle Kaban - betamesosaprobic, Lower kaban - polysaprobic, which is completely matching the estimation ofexperts by ecological condition of Kaban lakes by method of biological indication [15].

RESUME

By results of research the anticipated result of identification model of aquatic organisms by proteins completely matches experimental results and completely match to estimation of experts on ecological condition of water bodies by method of biological indication. Therefore, identification of aquatic organisms can be conducted by marker proteins in the same way as by marker genes. Differently from marker genes that allow to identify organism separately taken from sample, aquatic organism identification by marker proteins allow to work with aggregation of organisms in the sample. Model of aquatic organisms by marker proteins can be used for estimation of ecological condition of fresh-water bodies by method of biological indication.

SUMMARY

New approach to aquatic organisms identification by marker proteins with application of contemporary methods of bioinformatics and molecular genetics would allow employees of nature protecting bodies to receive the accurate information about water body population quickly and, therefore, give an accurate timely estimation of water body ecological condition.

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