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Starch gel electrophoresis isozyme analysis

淀粉胶电泳同工酶分析

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Foreword

Annex A, Annex B, Annex C, Annex D and Annex E of this standard are all normative annexes.

This standard was proposed by the Ministry of Agriculture of the People's Republic of China.

This standard is under the jurisdiction of the National Technical Committee on Aquatic Products Standardization.

Drafting organizations of this standard: Ocean University of China and Yellow Sea Fisheries Research Institute.

Main drafters of this standard: Gao Tianxiang, Zhang Yan, Han Zhiqiang, Xiao Yongshuang and Zhang Hui.

1 Scope

The Chinese national method for reading the isozymes of aquatic animals on a starch gel. Isozymes are different molecular forms of the same enzyme, and because they differ in charge and in size they travel at different speeds through a gel under an electric field; the pattern of bands that results is, in effect, a genetic fingerprint of the individual and, taken

over many individuals, of the stock. Fisheries science uses that fingerprint to tell hatchery strains apart, to check that a broodstock has not lost its diversity and to verify the germplasm of cultured fish against the standard for the species. Horizontal starch gel electrophoresis remains the workhorse for this because it is cheap and because a single gel can be sliced and stained for several enzyme systems at once - but it only gives comparable results if the buffers, the gel, the current, the staining and the scanning are all done the same way. This standard fixes them: the reagents and materials, the apparatus, the sampling and storage of tissue and blood, the analytical procedure from homogenization through electrophoresis, staining, drying and scanning, the calculation of the proportion of polymorphic loci and of the observed and expected heterozygosity, and the rules for judging individual results and the sample population. Five normative annexes give the preparation of the gel buffers, the electrophoresis buffers, the starch gel, the staining buffer and the staining solutions. It is used by fisheries research institutes and germplasm testing centres.

This standard specifies the reagents and materials, the apparatus, the sampling, the analytical procedure and the judgement of the results for starch gel electrophoresis isozyme analysis.

This standard is applicable to the analysis of the isozymes commonly examined by horizontal gel electrophoresis in common freshwater and seawater aquatic organisms.

2 Normative references

The provisions of the following documents become provisions of this standard through reference in this standard. For dated references, subsequent amendments (excluding corrections) to, or revisions of, any of these publications do not apply to this standard; however, the parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions. For undated references, the latest edition applies.

GB/T 18654.1-2008 Standard for germplasm testing of cultured fishes - Part 1: Rules for testing.

GB/T 18654.2-2008 Standard for germplasm testing of cultured fishes - Part 2: Sampling method.

3 Principle

Because isozymes differ in the charge they carry and in the size of their molecules, under the action of an electric field they migrate at different rates in the gel. After catalysis, staining and scanning, the zymogram bands of each isozyme, their number and their absorbance are obtained, and by comparing and analysing them the genetic characteristics of the fish are determined.

4 Reagents and materials

Except where otherwise stated, all reagents are of analytical grade or better, and the water is distilled water, deionized water or water of equivalent purity.

The clause lists the substances used: hydrolysed potato starch; the organic acids and their salts, namely citric acid, lactic acid, malic acid, lithium lactate, ethylenediaminetetraacetic acid and its disodium salt, disodium hydrogen phosphate and sodium dihydrogen phosphate; coenzyme I and coenzyme II; the stains and detection reagents, among them phenazine methosulfate, nitro blue tetrazolium chloride, trisodium isocitrate, sodium 6-phosphogluconate, 6-phosphoglucose, sorbitol, Fast Blue RR salt and the alpha-naphthyl and alpha-glycerophosphate substrates; ethanol, acetone, glacial acetic acid, hydrochloric acid, sodium hydroxide, heparin, magnesium chloride and 3-aminomethyl-morpholine.

It also lists the buffers and the gel that have to be made up rather than bought: the gel buffers TC-7.0, TC-8.0, CAPM-6.0 and CAPM-7.0, prepared as in Annex A; the electrophoresis buffers TC-7.0, TC-8.0, CAPM-6.0 and CAPM-7.0, prepared as in Annex B; the starch gel, prepared as in Annex C; the staining buffer, prepared as in Annex D; and the staining solutions of several common isozymes, prepared as in Annex E.

5 Apparatus

The clause lists the equipment required: a micro-homogenizer; a high speed refrigerated centrifuge of the stated speed; a multi-purpose electrophoresis unit; a vacuum pump; an electric furnace or a microwave oven; a gel slicer; multilayer filter paper; a laser scanner; a pH meter of the stated accuracy; gel moulds; staining boxes; a refrigerator and a low-temperature freezer; a constant-temperature incubator; an analytical balance and an electronic balance of the stated accuracies; and photographic equipment, namely a digital camera.

6 Sampling

6.1 Sampling of the samples. Carried out in accordance with GB/T 18654.2-2008.

6.2 Taking of the sample. A portion of tissue is taken from the sample and kept in the low-temperature freezer; for blood samples, heparin is added as an anticoagulant and the sample is kept refrigerated ready for use.

7 Analytical procedure

7.1 Preparation of the analytical sample. Before electrophoresis a portion of the sample is placed in a centrifuge tube with an equal volume of distilled water or of extraction solution and ground in an ice bath; the ground mixture is centrifuged in the refrigerated centrifuge until the supernatant is clear, and the supernatant is taken and kept frozen for loading. For

blood samples, the serum is loaded after separation.

7.2 Preparation of the gel. As given in Annex C.

7.3 Electrophoresis. A slit is cut in the gel at about one third of the electrophoresis distance from the edge; a piece of filter paper of suitable size soaked in the sample extract of 7.1, or soaked directly in juice from the frozen muscle, is inserted into the slit. After loading, electrophoresis buffer is added to the electrophoresis tank, filter paper bridges are used, and the run is carried out at constant current under refrigeration, the time depending on the species.

7.4 Staining and fixing. After electrophoresis the gel is sliced horizontally with a gel bow into thin slices and placed in staining boxes; the prepared staining solution is poured in and the boxes are stained in the constant-temperature incubator. When the colour has developed fully, the reaction is stopped with glacial acetic acid solution. The preparation of the staining solutions for the several enzymes is given in Annex D and Annex E.

7.5 Drying of the gel slices. The stained gel is placed on a sheet of glass paper of suitable size, pressed and sealed, air dried, numbered and kept.

7.6 Photography and scanning. The gel and its zymogram bands are photographed with a close-up lens, or the electrophoretic bands on the air dried slices are scanned with the laser scanner.

7.7 Analysis of the results. 7.7.1 Enzyme loci and allele analysis: from the structural composition of the enzyme and from the features the isozyme shows in the tissue, the coding gene locus of each isozyme, the polymorphic loci and the allele frequencies are determined. 7.7.2 Population genetic characteristics: the proportion of polymorphic loci, the observed mean heterozygosity and the expected mean heterozygosity are calculated by Formulae (1), (2) and (3) from the number of polymorphic loci, the total number of loci examined, the number of alleles at each locus and the frequencies of the homozygous genotypes.

8 Judgement of the results

8.1 Judgement of the result for an individual. Carried out in accordance with 6.1 of GB/T 18654.1-2008. All the measured results are compared one by one against the standard: those that conform to it or approach it are judged conforming, and those that do not conform to it, or that differ markedly from what the standard prescribes, are judged non-conforming.

8.2 Judgement of the sample population. From the results of the judgement in 8.1, the percentage of conforming items among the samples examined is calculated and expressed as a percentage.