

The resctriction endonucleases detection in colonies of microorganisms Streptomyces and Nocardia

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A simple technique is proposed for detection of restriction endonucleases in bacteria Streptomyces and Nocardia. The analysis was performed directly in the cells cultivated on Petri dishes and collected from colonies by an inoculation loop. The cells were treated with lysozyme, EDTA and Triton X-100. The lysates were tested for restriction endonucleases. The technique enables todetect the enzymes Nco I, Not I, Nru I I, Sfr 3031, and Sfi I in the lysates of corresponding strains-producers.

A wide use of restriction endonucleases in genetic engineering works resulted in a growing interest of researchers to the properties of these enzymes and methods of their production and detection [1]. The new restrictases discovery allows to widen the list of enzymes used in practical work and to replace the known producers of restriction endonucleases by more technological ones. A detection of the restriction enzymes in bacterial strains is a crucial step in the search for restrictase producing strains. These enzymes are part of restriction-modification system and may be determined by biological methods which are, however, quite difficult and need a special phages collection [2].

Lately, biochemical methods of the bacterial strains testing for the presence of site-specific DNase activity became widely used [3] [4-6]. These methods are based on preparation of clear lysates of bacterial cells and direct analysis of their enzymatic activity. These methods differ mainly in bacterial cells destruction. Among them there is a screening method that does not require preliminary cultivation of bacteria in a liquid nutrient medium and that significantly simplifies the detection procedure [3].

Based on the proposed method [3], we detected a number of new microorganisms- producers of restriction endonucleases, which refer to the genera *Bacillus*, *Micrococcus*, *Paracoccus*, *Pseudomonas*, *Vibrio* and some other genera of gram-negative and gram-positive bacteria [7], however, the proposed method does not allow testing strains referring to actinomyces for the presence of restrictases [3]. Other proposed biochemical methods have the same disadvantage [4-6].

This work describes a modified and even more simplified method for testing restriction endonucleases producing strains, which allows detection of restrictase activity in actinomycete cultures (*Nocardia* and *Streptomyces*) as well as *Staphylococcus*.

EXPERIMENTAL PART

Methods

The strains *Staphylococcus saprophyticus* B6 (B-4069), *Streptomyces fimbriatus* (S-750), *Streptomyces fradiae* (S-866), *Nocardia rubra* (B-3043), *Nocardia corallina* (B-613), *Nocardia otitidis-caviarum* (S-777) were obtained from the collection of industrial microorganisms of All-Union Research Institute of Genetics (Moscow).

Lysozyme type A (NPO "Biolar", Olaine), phage λ DNA (NPO "Ferment", Vilnius), Triton X-100 ("Serva", FRG), ethidium bromide and agarose type I ("Sigma", USA) were used in the work, pDKR 85 DNA [8] was isolated as described in [9]. The other reagents were home-produced, chemically pure, high purification degree.

The bacterial strains were plated on 1.5% agarized medium containing 35 g/l of sprat hydrolysate and grown for 2 days at 30°; 1–3 mg of cells from a Petri dish was suspended by pipetting into 100 μ l of a mixture containing 10 mM tris-HCl, pH 8.0, 1 mM EDTA, 0.1% Triton X-100 and freshly prepared lysozyme at the concentration of 1 g/l. The mixture was incubated for 3 h at room temperature and periodic suspending by pipetting. Then 10 μ l of solution containing 1 M tris-HCl, pH 8.0, 0.5 M KCl, 0.1 M $MgCl_2$ was added to each tube and samples were thoroughly mixed. Incubation mixtures were divided into two equal parts, and 3 μ l of phage λ DNA or plasmid (at the concentration of 0.5 mg/ml) was added to one half of the incubation mixture. Then two times dilutions of the obtained reaction mixture were prepared using a buffer of the following composition: 0.1 M tris-HCl, pH 8.0, 50 mM KCl, 10 mM $MgCl_2$, 50 μ g/ml of phage λ DNA or plasmid. Incubation was performed for 3 h at 37°. The reaction products were subjected to electrophoresis in 1% agarose gel. Electrophoresis buffer contained 89 mM tris, 89 mM H_3BO_3 , 2 mM EDTA, pH 8.0 and ethidium bromide at the concentration of 1 mg/l. Electrophoresis was performed at 10 V/cm for 1 h. The gels were photographed in ultraviolet light with a "Zenit" camera through a red filter.

RESULTS AND DISCUSSION

Previously we described a simple biochemical method of the bacterial strains testing for the presence of site-specific DNases including the stage of clear lysate preparation by centrifugation [3]. However, this method does not allow determining restriction endonuclease activity in some bacterial strains.

Fig. 1 presents the data of the restriction endonuclease activity determination in the strain *Staphylococcus saprophyticus* B6 at different schemes of cell destruction. As Fig. 1 shows, the preparation of clear lysate by centrifugation followed by incubation with phage λ DNA according to the previously described technique [3] does not allow detecting the presence of restrictase activity. However, excluding the centrifugation stage and the crude cell lysate testing provide a clear digestion picture at the same time of cells treatment (20 min at room temperature). Thus, detection of restriction endonucleases activity in crude lysate is more preferable for some bacterial strains.

Fig. 1 Determination of restriction endonuclease activity in the strain *Staphylococcus saprophyticus*B6. 1, 3, 5 - phage λ DNA; 2, 3 - crude lysate; 4, 5 - clear lysate (cleared by centrifugation).

This is possible because of some reasons. First, it might be associated with intracellular localization of restriction endonuclease. Centrifugation in course of clear lysate preparation may result in a significant decrease of enzyme's concentration in supernatant as compared with a pellet. Besides, cells lysis is actually going on in the course of incubation with the substrate DNA, which also influences the depth of the DNA cleavage.

However, the use of the cells crude lysates does not allow detecting restrictase activity in actinomycetes strains. For example, testing a crude lysate of the strain *Nocardia rubra*, producer of *Nru* I restrictase, doesn't detect the enzyme activity (data is not given).

To develop a biochemical method of testing actinomycete cultures for the presence of restriction endonucleases, we changed the conditions of bacterial cells lysis by increasing the concentration of lysozyme to 1 g/l as described in the "Methods", and the time of lysis and subsequent incubation with DNA to 3 h. In addition, we used the method of lysate dilution for more reliable detection of restrictase activity.

Fig. 2 presents the results of detection of *Ssr* B6 I, *Nru* I, *Nco* I, *Sfr* 303 I restriction endonucleases in crude lysates of the strains *Staphylococcus saprophyticus* B6, *Nocardia rubra*, *Nocardia corallina* and *Streptomyces fradiae* 303, respectively. A characteristic digestion picture is clearly observed for all four enzymes under conditions indicated in "Method". For the most of restriction enzymes a digestion pattern is getting better at the dilution of bacterial crude lysates.

Fig. 2 Testing the restrictases *Ssr* B6 I (a), *Nru* I (b), *Nco* I (c), *Sfr* 303 (d) from bacterial strains *Staphylococcus saprophyticus* B6, *Nocardia rubra*, *Nocardia corallina* and *Streptomyces fradiae*303, respectively. 1,2 - crude lysate, 2 - 6 - phage λ DNA. Crude lysat dilutions: 3 - 1/2; 4 - 1/4; 5 - 1/8; 6 - 1/16.

The search and detection of restriction endonucleases in actinomyces seem especially important in connection with the need for large-block fragmentation of DNA. By the present day, all known restriction endonucleases recognizing eight-bases nucleotide sequences (and because of this possessing rarely occurring recognition sites on prolonged DNA molecules) have been detected in microorganisms of the genera *Nocardia*, *Streptomyces*, *Frankia* [7].

Fig. 3 presents the data of testing the restrictases *Sfi* I and *Not* I in bacterial strains *Streptomyces fimbriatus* and *Nocardia otitidis-caviarum*, respectively, with the proposed method. Linearized plasmid pDKR 85 DNA containing recognition sites of the restrictases *Not* I and *Sfi* I was used as a substrate.

As Fig. 3 shows, the proposed method allows detection of restrictase activity in these strains, and, in our opinion, this method of restrictase testing may be used in the search for new restriction endonucleases.

Fig. 3 Detection of the rare-cutting restriction endonucleases *Sfi* I (a) and *Not* I (b) on plasmid pDKR 85 linearized by *Vsp* I restriction endonuclease. 1,2 - crude lysate, 2 - 5 - pDKR 85 DNA cleaved by *Vsp* I. Crude lysat dilutions: 3 - 1/2; 4 - 1/4

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