A PLASMID COMPLEX IN CELLS OF A BACTERIOCIN-PRODUCING STRAIN OF STAPHYLOCOCCUS

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KEY WORDS: staphylococci; plasmids; bacteriocin production; restriction.

Among the staphylococcal plasmids those which have received least study are plasmids controlling the bacteriocin-producing properties of these microorganisms. Suggestions that such plasmids exist have been made chiefly on the basis of data on their elimination from cells by acridine dyes or by other physical and chemical agencies [3, 4], and also on the identification of a plasmid controlling synthesis of bacteriocin and of exfoliative toxin simultaneously in *Staphylococcus aureus* of the second phage group.

The aim of this investigation was to determine the plasmid content of a bacteriocin-producing strain of *Staph. epidermidis* NJ and its variants.

EXPERIMENTAL METHOD

Strain *Staph. epidermidis* NJ 17 [1] and its plasmid-free variant, in which the plasmid of bacteriocin production was eliminated by culturing the cells at 44°C, were used. From the plasmid-free cells of this strain a spontaneous mutant resistant to 250 μg/ml of tetracycline was isolated. Resistance to 10^{-4} M cadmium ions was transmitted to this mutant in a mixed culture from strain NJ 17.

Plasmid DNA was isolated by the following method. Cultures of the strains used were grown in 30 ml of nutrient broth at 37°C with shaking for 18 h. The cells were harvested by centrifugation (5000 rpm, 30 min, 0°C), washed off with TES buffer, and resuspended in 3 ml of the same buffer. The cells were lysed by the addition of lysostaphin at 37°C for 1.5-2 h. After the beginning of lysis the samples were placed in an ice bath for 30 min, after which they were centrifuged (17,000 rpm, 40 min, 0°C). The supernatant was treated with pronase E in a final concentration of 500 μg/ml, dissolved in TEM buffer and heated for 2 h at 37°C, and also with 0.1 volume of 3 M sodium acetate and 2 volumes of rectified spirit. The samples were kept overnight at 4°C, then centrifuged (6000 rpm, 30 min, 0°C). The residue was treated with 1.5 ml of TES buffer and preparations kept at 4°C overnight.

The resulting preparations were analyzed for the presence of plasmid DNA by electrophoresis in agarose gel. For this purpose 0.9% agarose (type II Medium EEO, from Sigma, USA), dissolved in buffer of the following composition: Tris-HCl - 0.04 M, $CH_3COONa \times 3H_2O$ - 0.02 M, Na_2EDTA - 0.002 N, NaCl - 0.018 M, pH 7.5, was used. Ethidium bromide was added in a concentration of 1 μg/ml. Electrophoresis was carried out for 18 h. The samples were studied and photographed in UV light.

Treatment with restriction endonucleases Bgl II, EcoRI, and Sal I was carried out in a total volume of 50 μl. For Sal I and Bgl II a mixture of the following composition was used: Tris-HCl - 8 mM, $MgCl_2$ - 6 mM, Na_2EDTA - 0.2 mM, NaCl - 150 mM, pH 7.6, plasmid DNA 40 μl, restriction endonuclease 5 μl. For EcoRI the following mixture was used: Tris-HCl 0.04 M, $MgCl_2$ - 0.006 M, 2-mercaptoethanol 0.006 N, NaCl 0.06 M, pH 7.4. The duration of treatment of the preparations of plasmid DNA Sal I and Bgl II was 3 h, and EcoRI 1.5 h at 37°C. The reaction was stopped by heating the samples to 65°C for 5 min. Fragments of DNA of phage λ, treated with EcoRI and Sal I endonuclease, were used as standards of molecular weight.

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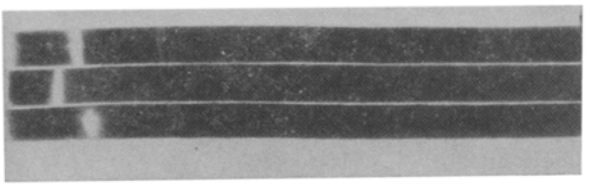

Fig. 1

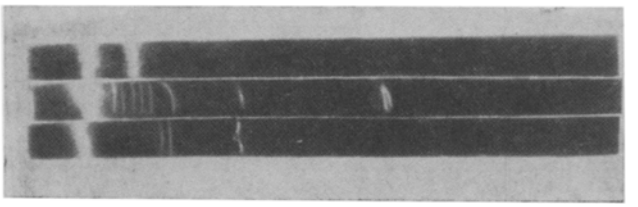

Fig. 2

Fig. 1. Electrophoresis of lysates of test strains untreated with restriction endonucleases. From left to right: strain NJ 17 (wild type); strain NJ 17 (plasmid-free variant); variant of strain NJ 17 resistant to cadmium.

Fig. 2. Electrophoresis of lysates of test strains treated with Sal I restriction endonuclease. From left to right: strain NJ 17 (wild type); variant of strain NJ 17 resistant to cadmium; DNA of bacteriophage λ.

EXPERIMENTAL RESULTS

Strain *Staph. epidermidis* NJ 17 was isolated from human skin and, in its morphological and biological properties, it was a typical representative of *Staph. epidermidis*. It possessed bacteriocin-producing properties, constitutive penicillinase activity, and was resistant to 10^{-4} M cadmium ions. On the basis of these findings it was concluded that the cells of this strain contained a plasmid complex.

The results of electrophoresis of lysates tested for the presence of plasmid DNA are illustrated in Fig. 1. Bands of plasmid DNA can be seen on the gel after electrophoresis of the original strain NJ 17. In the variant which had lost its bacteriocin-producing properties and resistance to cadmium ions, no bands of plasmid DNA can be detected. This variant, like the original strain, it will be noted, had the ability to produce constitutive penicillinase. The gene responsible for synthesis of this enzyme is thus located on the chromosome. Electrophoresis of a mutant of the variant of strain NJ 17 with acquired resistance only to cadmium ions likewise revealed bands of plasmid DNA. This is evidence in support of the view that the wild-type strain NJ 17 contains a plasmid complex consisting of at least two plasmids: the plasmid of bacteriocin production and the plasmid of resistance to cadmium ions.

To determine the molecular weight of the plasmids of the test strains, plasmid DNAs were treated with restriction endonucleases EcoRI, Sal I, and Bgl II. A segment of the gel from an experiment in which plasmid DNAs were treated with the Sal I enzyme is illustrated in Fig. 2. On the extreme right of the gel two fragments of DNA of phage λ with molecular weights of 20.7 and 9.8 megadaltons respectively can be seen. On the extreme left of the gel three bands corresponding to fragments with molecular weights of 1.99, 1.77, and 0.16 megadaltons can be seen. Evidently because of the presence of the plasmid complex in the wild-type strain NJ 17, restriction did not take place completely and not all fragments of the plasmid DNA could be found, so that it was impossible to determine the accurate molecular weight of the plasmids.

The next gel shown in Fig. 2 relates to analysis of DNA of the plasmid of resistance to cadmium ions. In this case at least eight bands can be seen with molecular weights of 14.13, 8.90, 5.01, 1.58, 0.19, 0.04, 0.01, and 0.001 megadaltons. The molecular weight of the plasmid of resistance to cadmium was thus 29.861 megadaltons.

The gene for resistance to cadmium ions is known to be a possible component of the penicillinase plasmids, or it may be an independent plasmid [8, 9]. Since the molecular weights of the penicillinase plasmids differ considerably from one another [7, 10], like the molecular weights of other plasmids which include resistance to cadmium ions [2], it is impossible to compare the plasmid of resistance to cadmium of strain NJ 17 with other plasmids described in the literature.

To sum up the results of this investigation it can be concluded that cells of strain *Staph. epidermidis* NJ 17 possess the gene of constitutive penicillinase, which is located on the chromosomes, and a plasmid complex consisting of the plasmid of resistance to cadmium ions, with a molecular weight of 29.861 megadaltons, and the plasmid of bacteriocin production, the molecular weight of which cannot be precisely determined until further investigations have been made.

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DETERMINATION OF THE NUMBER OF T AND B LYMPHOCYTES IN GUINEA PIGS

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Determination of T and B lymphocytes and of subpopulations of these cells is an essential condition for the study of the immune status of an individual. Guinea pigs are widely used for models of pathological processes, or the study of the action of biologically active substances, and so on. Recently data have been published on the property of the T lymphocytes of these animals of fixing rabbits' erythrocytes [8]. However, there is no information of the optimal conditions for formation of E rosettes by T lymphocytes of guinea pigs. There are likewise no data on whether these animals possess a fraction of "active" T lymphocytes, able to form rosettes directly after the addition of a suspension of xenogenic erythrocytes to them.

To determine β-lymphocytes of guinea pigs, the method of detecting receptors for C_3 complement on their surface was used [7, 8]. The effect of the concentration of complement, the class of antibodies used to form the antibody–complement complex, and centrifugation of a mixture of erythrocytes sensitized by this complex and lymphocytes on the course of this reaction likewise was not studied.

The aim of the investigation was to study the basic parameters of the method of determination of T and B lymphocytes of guinea pigs and the "active" fraction of T lymphocytes, and also to study the distribution of T and B lymphocytes *in vivo*.

EXPERIMENTAL METHOD

To obtain a suspension of lymphocytes the lymph nodes and bone marrow of guinea pigs weighing 250–300 g were placed in Hanks' solution, teased with dissection needles, and the suspension was filtered through four layers of Kapron gauze. The cells were washed 3 times with Hanks' solution for 10 min at 200g, resuspended in medium 199, and counted in a Goryaev's chamber. The viability of the cells was determined in the test with trypan blue. Before washing off, the bone marrow cells were treated with 0.85% ammonium chloride solution. Blood was collected by cardiac puncture, mixed with heparin (25–30 Units/ml blood) and diluted with Hanks' solution in the ratio of 1:3. The lymphocytes were isolated by the method in [5] and a suspension containing 2.5×10^6 cells/ml was prepared.

Erythrocytes were obtained from a rabbit and a sheep as follows. Blood was collected in an equal volume of Alsever's solution. The rabbit's erythrocytes were kept at least 4 days before the experiment. They were then washed 3 times before use and suspensions of rabbit's (10^8 cells/ml) and sheep's erythrocytes (5×10^8 cells/ml) were prepared.

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