LAB WORK 14

Lab Exercise 4.Staphylococcus phage-typing.

Methodical instructions: Bacteriophages are used for the diagnostics of infective diseases,determination of specific appliance of agent,identification of bacteria from environment. Phage-type allows to realize intraspecific differentiation of bacteria. Since phage-typoing characterization of bacterial strains is stable enough,it's extremely important for epidemiological analysis. Using phage labeling,it's impossible to establish links between individual cases and identify the sources of infection, ways of its expansion.

Widespread in the study received a diagnosis of staphylococcal infections of staphylococcal bacteriophages for coagulase positive staphylococcus isolated from human (International dialing - England) and from cattle (Davidson). These sets contain 23 and 7 types of bacteriophages. Typing is carried by all phage sets simultaneously.

Account and registration of results. Extent of Staphylococcus culture lysis is registred by the following scheme:

++++ - confluent(full) lysis;

+++ - semi-confluent (insignificant growth of culture in the zone of lysis);

++ - presence of more than 50 colonies of phage on the place of inserting the phage drops);

\+ - from 20 till 50 colonies of phage;

+- -less than 20 colonies of phage;

\- - full absence of lysis.

First 3 extent of lysis is called strong reaction. Extent of lysis (+,+-)-weak reaction.

Strain of Staphylococcus can be called typed, if one of the phage could give a strong reaction. If during the typing 1 TP were derived only weak reactions or lysis is absent, then this strain should be tested again with the same phages in 100 TP dilution.

Staphylococcus can with several phages, which helps to identify phage-puzzle to each strain. If the strain identify one phage-puzzle or has difference to one or two strong reactions, they will be identical.

Procedure:

1. Lyophilized contents of each ampuls with a typical phage was dissolved in 1 ml of Hottinger broth, Martin or beef- broth with 0.4 % glucose and 0.02% calcium chloride - the main dilution 10^{-1}.
2. Next, prepare dilutions corresponding to 1 dilution test (TP) and TP 100.
3. Daily agar culture of the test strain is inoculated into a test tube with a beef- broth (you can use Hottinger or March broth), pH 7.2-7.4 , and grown at 37 ° C till the appearing of visible turbidity (3- 5h).
4. Pour into Petri dishes 1.2% of Hottinger agar on fresh meat water with 0.4 % glucose and 0.02% calcium chloride (the latter is added directly to the molten medium ready before filling the dishes) .
5. Dishes with cooled agar is dried for 40-60 minutes at 37^0C. With the help of pasteur pipette irrigate the surface of agar.
6. Excess of culture is deleted and dried for 30-40 minutes at 37^0C.
7. Bottom of the seeded dish is divided in the correspondence of the quantity of used phage. Drop of corresponded phage should be inserted into the seeded medium.
8. After the drying of the drops of the phage, dishes should be turned over and incubated 18-20 hrs at 30 C or 5-6 hrs at 37 C and stayed in room temperature for 18-20hours.

Equipment:

- Cultures of microorganism on solid and liquid mediums

- Beef-extracted broth
- Beef-extracted agar
- Hottinger's broth
- Petri dishes Disinfectant tray
- Inoculation loop
- Burnerflame
- Spatula
- Bacterial pipette