New Method for Detecting Bacteriocin Production

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Bacteriocin production can be easily detected by using both sides of the agar contained in a standard petri dish.

Many methods for the detection of bacteriocin production have been described. All of the usual techniques are derived from those of Gratia (2) and Fredericq (1) and are based on the fact that bacteriocins can diffuse in solid or semisolid culture media, which are subsequently inoculated with a suitable indicator strain.

To ensure the sterility of the agar surface on which the indicator strain has to be inoculated, bacteriocin-producing strain is removed or killed by chloroform vapors before proceeding to the next step. If viable bacteria remain on the agar surface, their further growth might partially or totally mask the inhibition areas of the indicator strain.

Killing the bacteria of the producer strain does not allow the use of plastic petri dishes because chloroform vapors attack plastic. Residual chloroform in the culture medium insufficiently ventilated after exposure to vapors can give erroneous results.

The method described by Nicolle and Prunet (3) does not necessitate the killing or removing of the producer strain, which is inoculated under the surface of a truncated agar slant with a straight wire and then incubated for 2 days. On the third day, the indicator strain is inoculated on the surface of the slant. After incubation, the results can be recorded, by the presence or lack of growth of the indicator strain. This technique, requiring one test tube for every single producer or indicator strain, is therefore not very practical for screening purposes.

The new technique described here is based on the fact that diffusion of bacteriocins (like any other antibiotic) is tridimensional. The substance sufficiently ventilated after exposure to vapors can give erroneous results.

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All colonies of a *P. aeruginosa* strain are pyocin producers. can, therefore, be detected on the reverse side of the agar. This side will then serve as culture surface for the indicator strain.

The procedure is as follows. The producer strain is inoculated on the surface of nutrient agar in the usual way (spot inoculation, single colonies, or strip). After suitable incubation, the agar is detached from the edges of the petri dish with a sterile spatula. The plate is then inverted, and the petri dish is tapped sharply on the bench so that the agar disc falls into the lid. The sterile surface (previously at the bottom of the dish) is now uppermost in the lid, and the indicator strain can be inoculated. After incubation, zones of inhibition are clearly visible.

By using this technique, 25 *Streptococcus faecalis* strains and 143 *Pseudomonas aeruginosa* strains have been tested for bacteriocin production and bacteriocin sensitivity.

Several applications of this method to pyocins are shown in Fig. 1, 2, 3, and 4.

As there is no direct contact between the producer and the indicator strains, phage particles cannot reach the indicator bacteria. Therefore, no inhibition area due to phages can be formed, as is often the case with other methods where it is not always easy to decide whether a zone of confluent lysis is due to phages or to bacteriocins.

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