A Comparative Study of Special Agar Medium (Redmond) and Simple Agar Medium for the Phage-typing of Mycobacteria

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Phage-typing is an important technique for classifying mycobacteria and optimum conditions for growth are required to bring the organisms into the logarithmic phase of growth needed for their exposure to phages. The quality of the media used for growing the strains selected for typing is therefore important.

Comparative trials on the propagation of 3 different mycobacterial phages (MyF₁-P₁₁₈; MyF₂-P₁₁₉, both isolated in Prague, and GS₄E, supplied by W. B. Redmond, Atlanta, Ga., USA) on ATCC 607 and S₁P (scotochromogen) mycobacterial strains grown on RVA (Redmond) medium and on simple N-1 agar, with and without albumin, were undertaken. The results showed that the Redmond medium is very sensitive for growing atypical strains but not for multiplying phages. The lytic plaques were less developed on this medium and the metabolic activity of the phages—as shown by the absence of a positive tellurite phenomenon—was impaired. Further comparative trials with slowly growing mycobacteria are in progress.

The problem of standardizing media is important for the phage-typing of mycobacteria, as well as for isolating the organisms and testing their sensitivity to drugs. The ideal solution to this problem would be to have a chemically defined synthetic medium that could be prepared in lyophilized form and stored for a long period without loss of sensitivity.

Extensive studies undertaken in this field at the WHO Reference Laboratory, Prague, led to the development of a lyophilized concentrated liquid medium that has proved to be as sensitive as Löwenstein-Jensen medium for the isolation of mycobacteria from pathological specimens (Šula, 1963). This medium, together with others—including Middlebrook’s 7 H9 and 7 H10, and the Dubos medium of Redmond—were tested for their suitability for phage-typing. As a result, a new medium (RVA) was developed and used successfully in the first set of co-operative trials conducted by the WHO Reference Laboratory, Prague, and the Veterans’ Administration Tuberculosis Research Laboratory, Atlanta, Ga., USA (Redmond & Ward, 1966).

For the second set of co-operative trials, a lyophilized medium was prepared by Redmond and tested in Prague in parallel with a simple N-1 agar medium, with and without albumin. The main advantage of the Redmond medium is the simplicity of its preparation; special chemicals and biological ingredients, that are not always obtainable, are not required.

MATERIAL AND METHODS

Media

Redmond’s special medium. Special RVA agar base (DIFCO, lot 606-630) reconstituted in Prague. First, 28.9 g of this agar were dissolved in 975 ml of distilled water containing 8 ml of glycerol, at 56°C. After this solution had cooled to 47°C, 25 ml of oleic acid albumin solution of the following composition were added:

- Bovine albumin fraction V 3.5 g
- N/20 sodium hydroxide + oleic acid (0.12 ml oleic acid in 10 ml N/20 NaOH) 5.0 ml
- 2.5 N sodium hydroxide 0.35 ml
- Water 20 ml

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Sterilization by Seitz filtration was performed, and 1 litre of special RVA agar base, reconstituted as stated above, was added.

*Synthetic N-1 medium.* The composition of this medium is as follows:

- Potassium mono-hydrogen phosphate: 1.5 g
- Magnesium sulfate: 0.5 g
- Asparagin: 1.5 g
- Glycerol: 5.0 g
- DIFCO agar base: 20.0 g
- Distilled water to 1000.0 ml

*Semi-synthetic N-1 medium.* This medium had the same composition as shown above, with addition of 0.2% of bovine albumin fraction V.

**Phages**

Strains MyF1-P/58; MyF2-P/59 (originally isolated at the WHO Reference Laboratory, Prague); GS4E (supplied by W. B. Redmond, Atlanta, Ga., USA).

**Strains tested**

ATCC 607 (supplied by P. Hauduroy, Centre International de Distribution de Souches et d’Informations sur les Types microbiens, Lausanne, Switzerland); scotochromogen S,P (supplied by G. Penso, Istituto Superiore di Sanità, Rome, Italy).

**PREPARATION OF CULTURES AND PHAGE STOCKS**

The strains ATCC 607 and S,P were subcultured in 5 ml (0.5 ml of inoculum) of Šula’s liquid medium and incubated at 37°C for 24 hours. These cultures were used for exposure to phages on Redmond and N-1 solid agar media.

Five ml of the 24-hour S,P culture were inoculated into 2 × 100 ml of Šula’s liquid medium and incubated for 4 hours. Afterwards 10 ml of MyF1-P/58 stock phage suspension were added. The culture was incubated at 37°C for 48 hours, and then passed through a sintered-glass filter. In the same way, MyF2-P/59 and GS4E stock phage suspensions were prepared using ATCC 607 and a mixture of ATCC 607 and H37Rv, respectively. The strain H37Rv was grown in liquid medium for 10 days.

**TESTING OF LYTIC ACTIVITY OF THE PHAGES**

Nine 10-fold dilutions of each phage stock in Šula’s liquid medium were prepared and 0.1 ml of each was inoculated with a pipette on to Redmond and N-1 agar media. The media were first flooded with the cultures of the respective strains, the excess liquid culture being removed by pipette, and the inoculated media left at 37°C for 4 hours. Afterwards they were seen to be spotted with the phages. The exposed cultures were read after 24-48 hours and the number of plaques was noted. The titres ranged between 10⁻⁸ and 10⁻⁹ particles.

**QUALITATIVE ANALYSIS OF THE PLAQUES BY THE TELLURITE PHENOMENON**

After formation of the plaques (24-48 hours), 3 ml-5 ml of 1‰ potassium tellurite (K₂TeO₃) were added to the culture, and the resultant black discoloration (tellurite phenomenon) was read after 4 and 24 hours at room temperature (20°C) (Šula & Šulová, 1965).

Lysis was produced in both media inoculated with ATCC 607 and S,P strains exposed to the above-mentioned phages. However, there were differences between the media as regards the qualitative and quantitative pattern of the lysis.

Phage MyF1-P/58 produced the best lysis in the strain S,P on the N-1 medium with albumin—almost identical with the lytic pattern observed in the same medium without albumin. The lytic plaques were well developed, sharply circumscribed against the growing culture, and surrounded by the typical halo of a positive tellurite phenomenon (Fig. 1).

On the other hand, the lysis observed on Redmond’s medium was not so marked. The plaques consisted of submiliary lytic spots which were not as well defined. The number of plaques was about the same as on the other media, but there was not the characteristic peripheral halo.

The differences in the lytic pattern observed in the Redmond and in the N-1 media are clearly demonstrated in Fig. 2. This shows a large zone of tellurite-negative culture bordering the lytic plaques in N-1 medium, which is almost absent from the Redmond medium.

The same lytic pattern was found with the ATCC 607 strain exposed to the phage MyF2-P/59 (Fig. 3) and to the phage GS4E (Fig. 4). Again, the plaques were better developed in the N-1 medium, with or without albumin, than in the Redmond medium.

**DISCUSSION**

In order to develop a medium suitable for the phage-typing of mycobacteria, certain conditions must be fulfilled; these are applicable to phages in
FIG. 1
LYTIC PATTERN PRODUCED BY PHAGE MyF-P/58 IN STRAIN S1P ON N-1 MEDIUM, WITH AND WITHOUT ALBUMIN, IN COMPARISON WITH REDMOND MEDIUM

a The 2 Petri dishes on the left side contain N-1 medium with albumin, the 2 dishes in the middle contain Redmond medium and the 2 dishes on the right side contain N-1 medium without albumin.

FIG. 2
LYTIC PATTERN AFTER THE TELLURITE PHENOMENON

a The Petri dish on the left contains Redmond medium, the dish on the right contains N-1 medium. There is a marked difference between the media as regards the lytic pattern.
FIG. 3
DIFFERENCES IN THE CHARACTERISTICS OF THE LYTIC PLAQUES PRODUCED IN STRAIN ATCC 607 EXPOSED TO PHAGE MyFi-P/59

The Petri dish on the left contains Redmond medium, the dish in the middle N-1 medium without albumin and the dish on the right N-1 medium with albumin.

FIG. 4
DIFFERENCES IN THE CHARACTERISTICS OF THE LYTIC PLAQUES PRODUCED IN STRAIN ATCC 607 EXPOSED TO PHAGE GS4E

The Petri dish on the left contains Redmond medium, the dish in the middle N-1 medium without albumin and the dish on the right N-1 medium with albumin. It can be seen that the general lytic pattern is the same as that in Fig. 3.
general. First, the micro-organisms must be in the logarithmic phase of growth. Secondly, the conditions must be favourable for the fixation, penetration and multiplication of the phages in the bacilli. In these circumstances, the conditions that will permit both multiplication and survival are much more difficult to achieve than for simple cultivation of mycobacteria. A medium that may be ideal for culturing mycobacteria will not necessarily allow the fixation, penetration and growth of phages, and vice versa. This has actually been demonstrated in our comparative trials with 3 different phages, 2 mycobacterial strains and 2 media.

From the analysis of the lytic pattern, it may be concluded that the Redmond lyophilized medium is ideal for multiplying the strains under study, since the amount of mycobacterial growth was more extensive on the surface of the agar, where it formed a thick membrane, than it was on the N-1 medium, where only a thin film was formed. On the other hand, the latter is more suitable for phage propagation. This was demonstrated by the larger size of the plaques and the greater metabolic activity of the phages, shown by a positive tellurite phenomenon which was almost absent from the Redmond medium.

Further comparative trials with slowly growing mycobacteria are in progress.

RÉSUMÉ

ÉTUDE COMPARATIVE D’UN MILIEU GÉLOSÉ SPÉCIAL (REDMOND) ET D’UN MILIEU GÉLOSÉ SIMPLE (N-1) POUR LA LYSOTYPIE DES MYCOBACTÉRIES

Au cours d’essais comparatifs, les auteurs ont étudié la propagation de trois mycophages différents (MyF₁-P/58; MyF₂-P/59, isolés à Prague; et GS4E, fourni par Redmond, Atlanta, Etats-Unis d’Amérique) sur les souches de mycobactéries ATCC 607 et S₁P (scotochromogène), mises en culture en milieu spécial de Redmond et en milieu gélosé simple N-1, avec ou sans albumine. Les résultats ont montré que le milieu de Redmond convenait parfaitement à la culture des souches atypiques, mais non à la multiplication des phages. Les plaques de lyse étaient moins développées dans ce milieu et l’activité métabolique des mycophages se trouvait freinée, comme l’indique l’absence d’un phénomène de zone positif après addition aux cultures de tellurite de potassium. De nouveaux essais comparatifs portant sur les mycobactéries à croissance lente sont actuellement en cours.

REFERENCES