observing the colors. Results are less satisfactory when the room temperature rises above 22°C.

REFERENCE

FUJITA, A., AND YOSHIOKA, S. 1923 A new medium favorable for pigment production by staphylococcus, also a contribution to the knowledge of pigment production. Japan Med. World, **3**, 47–51.

A MEDIUM ADAPTED TO THE BACTERIOPHAGE OF RHIZOBIUM LEGUMINOSARUM[1]

THRESSA CAMPBELL AND A. W. HOFER

Division of Bacteriology, New York State Agricultural Experiment Station, Geneva, New York[2]

Received for publication July 31, 1942

In a series of studies on soil bacteriophage, progress was found to depend upon the development of better technic in connection with the test-tube method of detecting bacteriophage. The medium M_5 of Laird (1932), for example, although generally regarded as satisfactory for the bacteriophage of *Rhizobium spp.*, was found to have certain disadvantages: It does not allow sufficient growth of *R. leguminosarum*, the pea nodule organism; the $CaCO_3$, added to favor development of lysis, causes a turbidity due to precipitated calcium salts on the wall of the tube, which sometimes makes it difficult to determine whether lysis has occurred; the light brown color of the yeast extract is sometimes a disadvantage for the same reason.

The first change made in this medium has been to follow the suggestion of Albrecht and McCalla (1937) to supply the needed growth factors in the form of sauerkraut juice instead of yeast extract. This furnishes a colorless medium, allowing even better growth of the bacteria in question than when yeast extract is employed.

The second change has been to replace the $CaCO_3$ and K_2HPO_4 of Laird's medium with calcium glycerophosphate. The latter contains both calcium and phosphorus, but unlike the simple calcium phosphates, is soluble at neutrality and at weakly alkaline reactions; its use, therefore, eliminates the objectionable haziness. It has the further advantage of supplying a small amount of available carbon; this promotes growth without the formation of sufficient gummy mate-

[1] Approved by the Director of the N. Y. State Agricultural Experiment Station for publication as Journal Paper No. 519, July 28, 1942.

[2] This investigation, supported by grants from the Urbana Laboratories, Urbana, Illinois, and the Cooperative G. L. F. Mills, Inc., Buffalo, New York, was carried on in cooperation with the Department of Agronomy of the New York State College of Agriculture.

rial to interfere with lysis, as may be the case if more carbon is furnished, e.g., in the form of sugar or a polyhydric alcohol. The formula recommended is:

Calcium glycerophosphate	1 g.
$MgSO_4$	0.2 g.
NaCl	0.2 g.
Sauerkraut juice	20 ml.
Distilled water	980 ml.
Reaction adjusted to	pH 7.6

As a result of these changes, greater turbidity is produced by the culture; and when lysis occurs, the clearing is more marked. Usually bacteriophage can be demonstrated by adding 100 g. soil containing the specific bacteriophage to 50 ml. of this medium which has previously been inoculated with the susceptible organism; the optimum incubation is 24 hr. at 30°C.

REFERENCES

ALBRECHT, W. A., AND MCCALLA, T. M. 1937 A new culture medium for rhizobia. J. Bact., **34**, 455–457.

LAIRD, D. G. 1932 Bacteriophage and the root nodule bacteria. Arch. Mikrobiol., **3**, 159–193.

SEMI-QUANTITATIVE DETERMINATIONS OF BACTERIOPHAGE IN SOILS[1]

ELIZABETH J. BOTTCHER AND A. W. HOFER

Division of Bacteriology, New York State Agricultural Experiment Station, Geneva, New York[2]

Received for publication July 31, 1942

The test-tube procedure using the medium of Campbell and Hofer (preceding note) is purely a qualitative test. It should be pointed out, however, that a quantitative test for bacteriophage in soil is desirable which would be applicable not only to those races lytic to *Rhizobium spp.* but also to those attacking other kinds of soil bacteria. The successful use of the Campbell and Hofer sauerkraut-glycerophosphate medium in studying the bacteriophage of the pea nodule organism suggested that this technic might be developed into a quantitative procedure.

Many procedures for obtaining bacteriophage from soil have been described in the literature, as summarized by Fuller and Vandecaveye (1942). None are quantitative, although Katznelson (1939) proposed a plate method to determine quantitatively the bacteriophage content of cultures. The method which is here described is designed to give a semi-quantitative comparison of

[1] Approved by the Director of the N. Y. State Agricultural Experiment Station for publication as Journal Paper No. 520. July 28, 1942.

[2] In coöperation with the Department of Agronomy, N. Y. State College of Agriculture.