## Synthetic media for maintenance and induction of lysogenic *Bacillus megaterium*

Studies on the development of bacteriophage in protoplasts of *Bacillus megaterium* (SALTON AND MCQUILLEN[1]) made it necessary to devise media for the lysogenic strain 899(1) (kindly provided by Dr. LWOFF). Two types of medium were needed—one for maintenance of the lysogenic character of the culture (continued subculture in unsuitable media can lead to loss of this property[2,3]) and another medium to confer what LWOFF[3] calls "aptitude", the condition of the culture in which treatment with an inducing agent such as ultra-violet light, hydrogen peroxide, *etc.*, causes lysis of the host cells and liberation of mature bacteriophages. A single medium may of course satisfy both requirements.

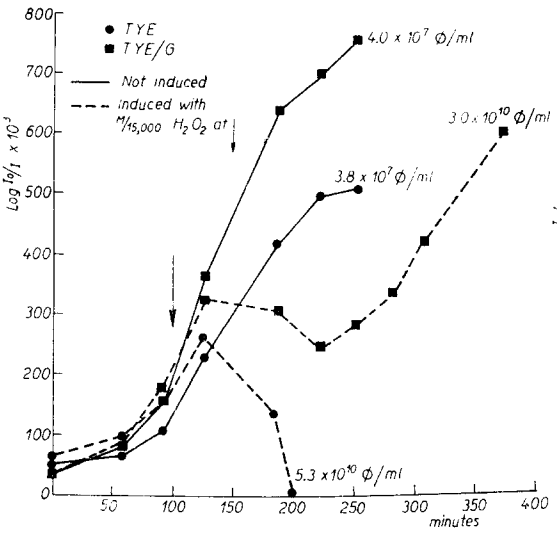
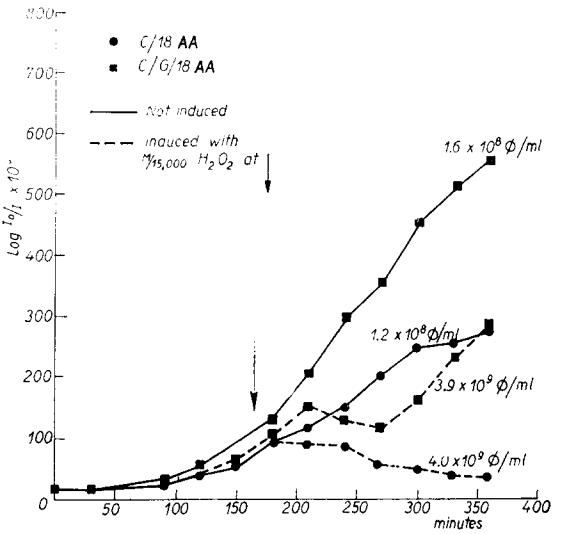



Fig. 1 A

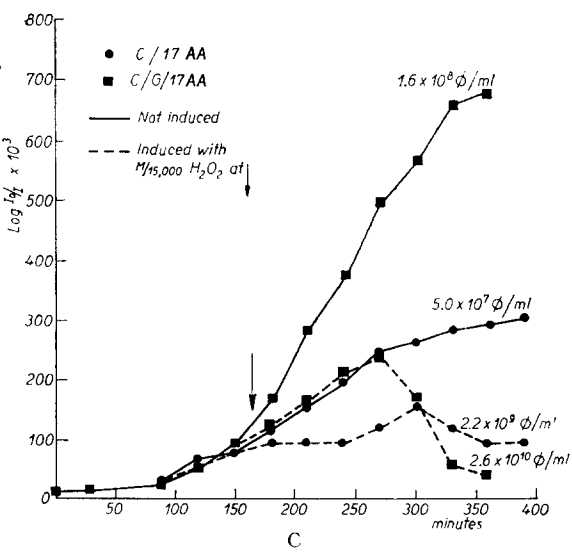



B

TABLE I

Components of media – g/litre

| *Inorganic salts solution "C"* | | *Amino-acid mixture "18AA"* |
|---|---|---|
| $NH_4Cl$ | 2 | DL-Alanine |
| $Na_2HPO_4$ | 6 | L-Arginine·HCl |
| $KH_2PO_4$ | 3 | L-Aspartic acid |
| NaCl | 3 | L-Cysteine·HCl |
| $MgCl_2 \cdot 6H_2O$ | 0.1 | L-Glutamic acid |
| $Na_2SO_4$ | 0.11 | Glycine |
| (Analar grade reagents) | | L-Histidine (base) |
| | | DL-Isoleucine |
| | | L-Leucine |
| *Glucose solution "G"* | | L-Lysine·2HCl |
| Glucose | 5 | DL-Methionine |
| Glucose sterilized separately | | DL-Phenylalanine |
| | | L-Proline |
| | | DL-Serine |
| | | DL-Threonine |
| | | DL-Tryptophan |
| | | L-Tyrosine |
| | | L-Valine |
| | | 0.05 g of each |




C

The media were made up in distilled water and referred to as C/G, C/G/18AA, *etc.*, according to which components were present. When only 17 amino-acids were used, cysteine was the one omitted. Cultures were aerated at 30° C.

Synthetic media have not in the past been found satisfactory for this lysogenic system[2,3]. However, the following medium C/G[4] (Table I) has been used for daily transfer of *B. megaterium* 899(1) and lysogeny has not been lost after 80 subcultures.

On the other hand, induction has never been brought about in C/G even with the further addition of trace elements and calcium. Transfer to a Bacto tryptone-Bacto yeast extract medium (TYE) immediately rendered the culture "apt" for induction. An equally effective but fully synthetic induction medium was found to be the basal salts solution, C, plus 18 amino-acids (50–400 $\mu$g/ml of each) and with or without 0.5% glucose (C/18AA or C/18AA/G). Omission of cysteine which might itself act as an inducing agent, did not impair the medium (C/17AA/G).

Figs. 1A, B and C show typical results. Various media were inoculated with a culture which had been transferred more than 20 times in C/G and when growth was vigorous, hydrogen peroxide (final concentration $M/15{,}000$) was added as inducing agent. After lysis had occurred, the phage titres of induced cultures were $5 \cdot 10^9$–$5 \cdot 10^{10}$/ml in both synthetic and complex media.

It is apparent that the property of lysogeny can be maintained for long periods of cultivation in a simple glucose/salts medium. It is also evident that induction can be achieved with the same dosage of inducing agent in glucose/amino-acids/salts medium as in complex media (contrast LWOFF[3]). Neither calcium ions nor trace elements improved the performance. The only divalent cation in the medium was magnesium at a concentration of 10 mg/litre.

KENNETH MCQUILLEN
M. R. J. SALTON

*Department of Biochemistry, University of Cambridge (England)*

[1] M. R. J. SALTON AND K. MCQUILLEN, in preparation.
[2] N. A. CLARKE, *J. Bacteriol.*, 63 (1952)187.
[3] A. LWOFF, *Bacteriol. Revs.*, 17 (1953) 269.
[4] K. MCQUILLEN AND R. B. ROBERTS, *J. Biol. Chem.*, 207 (1954) 81.

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## C-terminal amino-acid sequence of tobacco mosaic virus protein

When tobacco mosaic virus (TMV) is treated with carboxypeptidase, about 2,900 residues of threonine, and only threonine, are released per mole[1,2]. This remarkable finding is in accord with other observations[3] which suggest that each virus particle contains approximately 2,900 peptide-chain sub-units. However, the release of many threonine residues occurring in sequence and other interpretations are by no means excluded. A chemical attack on the protein would give definitive data on this point and might yield further information concerning the terminal sequence.

When the protein prepared from TMV was subjected to hydrazinolysis, according to AKABORI AND OHNO[4,5], and the DNP-derivative of the free C-terminal amino acid identified and determined by 2-dimensional chromatography[6], only threonine was found. The amount of threonine released from the protein, corrected for the losses inherent in the method, corresponded to 1 equivalent per 18,000 g (Table I). Thus, the C-terminal position of about 2,800 threonine residues and only threonine, was definitely established[7].

TABLE I

RELEASE OF C-TERMINAL REACTION PRODUCTS OF HYDRAZINOLYSIS

| *Hydrazinolysis time* | *TMV protein threonine* | *Dethreoninated TMV protein* | |
|---|---|---|---|
| | | *Alanine* | *Prolyl-alanine* |
| (h) | equivalents per 18,000 g protein* | | |
| 4 | — | 0.09 | 0.48 |
| 5 | 0.79 | 0.35 | 0.32 |
| 10 | 1.00 | 0.94 | 0.08 |
| 22 | 0.95 | 0.92 | trace |

* Corrected by factors of loss incurred during hydrazinolysis (10 h period), dinitrophenylation and extraction of 0.62, 0.63 and 0.63 for threonine, alanine and prolyl-alanine respectively. Most data are the averaged results of several runs, usually with about 0.5–1.0 $\mu M$ of protein.

When the protein isolated from enzymatically dethreoninated virus was reacted with hydrazine for 10 or 22 hours, only one free amino acid, isolated as the DNP-derivative, was found in near-stoichiometric amount. This amino acid was identified as alanine. In addition, a trace of an unknown