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ISOLATION OF NEW CYANOPHAGE An-1

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SINCE the discovery of a cyanophage in 1963[1], more than 100 types of cyanophages have been recorded in the past 25 years. These cyanophages have been broadly categorized into two main types—one infecting filamentous cyanobacteria and the other to unicellular or colonial forms[2]. During our studies on cyanophage infecting unicellular alga *Anacystis nidulans*, we have found a new type which is being reported in this communication.

During the present studies, four test organisms, viz. *Anacystis nidulans*, *Gloeocapsa* sp., *Gloeothece* sp. and *Synechococcus cedrorum* were selected for initial screening against 19 strains of cyanobacteria being maintained in the National Facility for BGA Collections. The screening was done by soaking 5 mm diameter sterilized filter paper discs in the culture

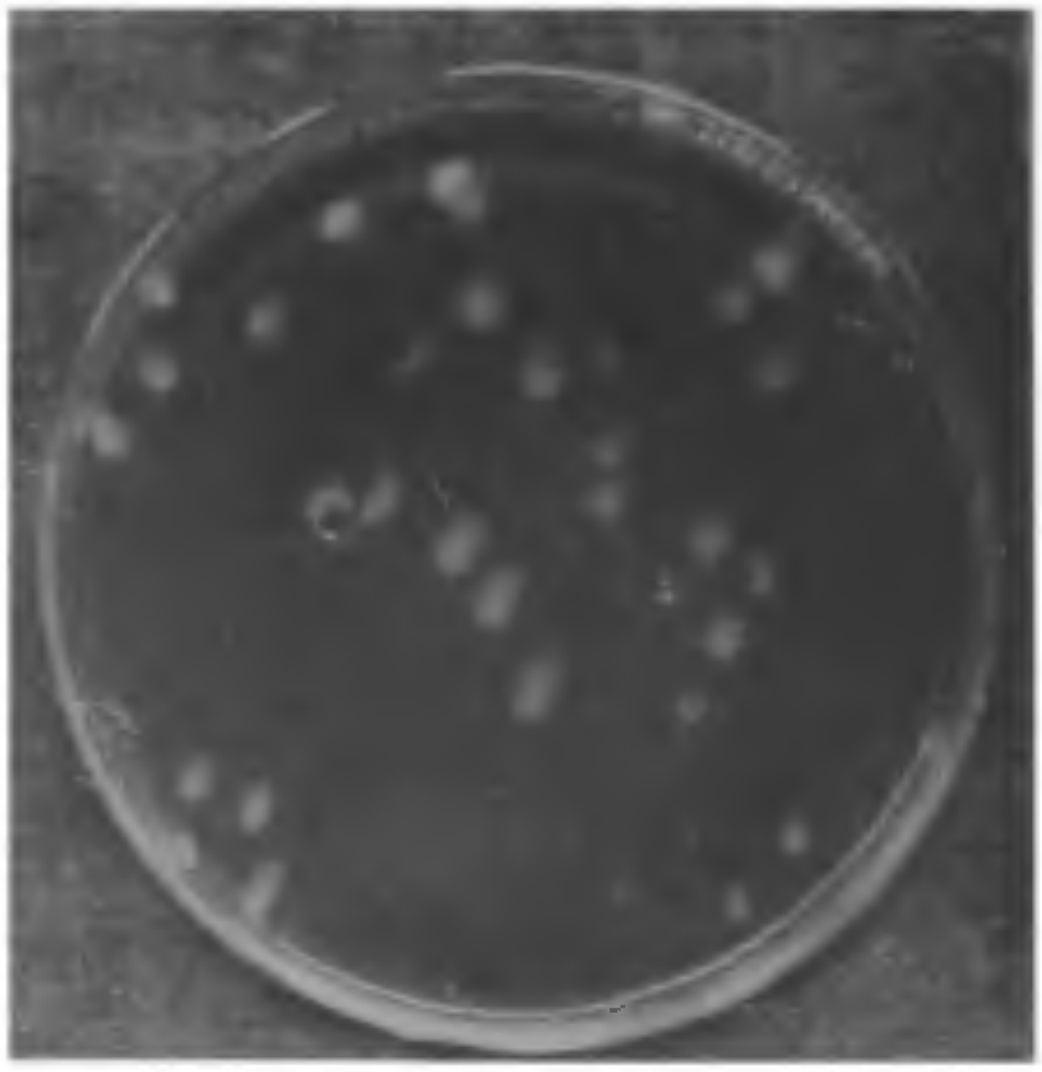

Figure 1. Plaques of An-1 on the lawn of *Anacystis nidulans*.

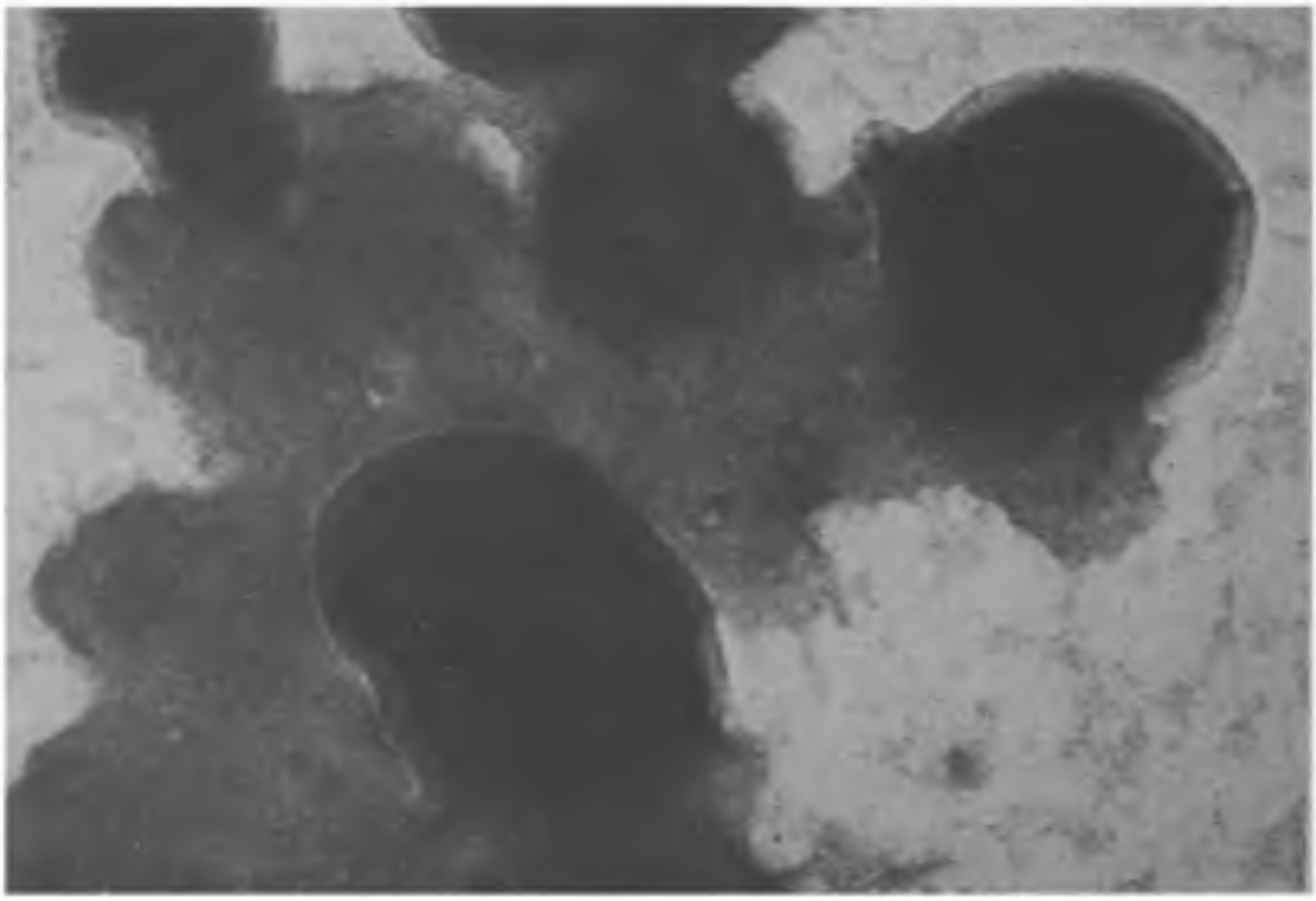

Figure 2. Head-tail type cyanophage An-1 specific to *Anacystis nidulans*.

filtrates and placing them on the lawn of test organisms.

It was observed that the growth of *A. nidulans* was inhibited and clear zones were formed. These zones were carefully scraped and inoculated to a suspension of *A. nidulans* in BG-11[3] medium. It was interesting to note that the suspension was lysed within four days. For purification, the lysate was treated with chloroform (0.2 ml/ml lysate) and passed through G-5 ultrafine sintered glass filters. This preparation was plated on the host. It was found that the phage produced typical oval to oblong plaques of 3–4 mm in diameter (figure 1). Individual plaques were further multiplied to build up the phage titre.

The high titre phage preparation was used for electron microscopy by the negative staining method. In the electron microscope, bodies resembling 'head-tail' type phage were observed. The head was hexagonal in shape with an average diameter of 71.1 nm. A short tail of 20 nm long was seen attached to one of its vertices (figure 2).

To determine the host range, 80 available strains of cyanobacteria were screened against this phage but none was lysed.

The present phage did not provide any evidence of tail fibres as reported in AS-1 another phage infecting *Anacystis* and *Synechococcus*[4]. In the morphology of head, the present phage differed from AS-1 and SM-1 being smaller than AS-1 and bigger than SM-1[5]. It also differs from these two phages on the basis of its tail length which is 12 times smaller than AS-1 but much larger than SM-1. On the basis of its morphology and host range, the present phage appears to be distinct from cyanophages reported so far and thus forms a new record. We propose to name this phage as An-1 to differentiate it from A-series phages infecting the species of *Anabaena*[6].

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IN VITRO MULTIPLE PLANTLET REGENERATION IN KNOL KHOL (*BRASSICA OLERACEA* L. VAR. *CAULORAPA*)

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BRASSICA comprises of several oil-seed and vegetable crop species. A recent review[1] on *in vitro* studies of the genera reveals that the response of various species ranges from simple callusing of the explant to plantlet regeneration from protoplast. Clonal multiplication has been achieved in two varieties of *Brassica oleracea*, viz. cabbage[2,3] and cauliflower[1] but no such report is available on Knol Khol, *Brassica oleracea* var. *caulorapa*. It is an important vegetable crop and some of the traits deserve genetic improvement. Since the application of biotechnological approach for plant improvement is easier in crops that are amenable to *in vitro* regeneration[4] an attempt was made to achieve this end point. The present paper describes a protocol for *in vitro* plantlet regeneration of *Brassica oleracea* var. *caulorapa*.

Seeds of *Brassica oleracea* var. *caulorapa* were surface-sterilized with 0.2% mercuric chloride for 10 minutes and then rinsed 3–4 times in sterile distilled water. Ten seeds were inoculated for germination in each flask containing semisolid agar medium (0.8%). These flasks were placed in a dark cabinet. After 14 days various explants were excised from the seedlings and inoculated in Murashige and Skoog's medium[5] supplemented with different concentrations and combinations of plant harmones. All cultures were maintained at 28 ± 2°C under 12/12 h photoperiod. Shootlets so obtained were placed in the rooting medium.

Responses of the hypocotyl and cotyledon explants to indole acetic acid (IAA) and 6-benzyl amino purine (6-BAP) are shown in table 1. The hypocotyl in all the media tested exhibits induction of callus without organogenesis. Cotyledons enlarge and produce callus at the lower side. Small roots also appeared after some time.

Cotyledons with stem apex were quite responsive to MS medium containing IAA + 6-BAP. Although induction of callus and organogenesis was observed in various ratios of IAA:6-BAP, profuse proliferation of shootlet was found in MS + 0.5 mg/l IAA + 5 mg/l 6-BAP (table 2). Following is the chronology of events in this medium.